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AN OUTLINE OF THE PROCESS OF ORGANIC EVOLUTION

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THE NATURE OF EVOLUTION

Organic evolution is descent with change. Present-day organisms differ from their ancestors of the past, and the differences between past and present organisms have come about slowly. The organisms now on the earth are related through descent, and the degree of similarity between them is an indication of the recentness of their common ancestry. Evolution is a process which results in dissimilarities between ancestral and descendent populations. It is a process of change, from generation to generation, in the genetic composition of species populations. The factors which bring about organic evolution have been in operation for many millions of years and are still operative, and some of them can be studied experimentally.

Certain features of the evolutionary process may be deduced from the data of paleontology and other fields. Evolutionary change in a species is usually very slow; the differences in organisms found in the fossil record have been occurring over millions of years. However, this process is not equally slow in all groups of organisms; it is in general slower in aquatic than in terrestrial organisms, and slower in more constant evironments. The changes which occur result in differences in physiology, structure (gain, loss, or modification of a structure, or a change in size), and behavior, and may result in a change of habitat by the organism. Evolutionary changes in organisms sometimes occur as the environmental conditions change; sometimes environmental changes are not accompanied by evolutionary changes in the organism, and it becomes extinct. Evolutionary changes in an animal may proceed in a given direction (for example, increase in size) for a long period of time (orthogenesis). On the other hand, a species may undergo evolutionary change even when living under constant environmental conditions. Evolution in isolated groups living under similar conditions often occurs in different directions, and the amount of difference which develops between them usually increases the longer they are isolated. Evolutionary changes in species generally proceed in the direction of increased specialization. On the basis of data from various sources it is often possible to determine the evolutionary changes that have occurred in the phylogeny of a particular present-day animal.

There are two more or less distinct phases of the evolutionary process, (1) the nature and sources of genetic change in the cells of the organism (sometimes spoken of as evolutionary statics), and (2) the factors which influence the trend or direction of evolutionary change in species populations (sometimes spoken of as evolutionary dynamics).

THE NATURE AND SOURCES OF GENETIC CHANGE

Changes in the evolution of a sexually reproducing animal must be genetic, otherwise they do not pass to succeeding generations. The Lamarckian hypothesis of the inheritance of acquired characters is not adequate to explain the origin of genetic changes. An organism cannot respond to environmental conditions by producing corresponding genetic changes. Somatic changes, that is, changes in the body of an organism but not in the germ plasm, which are induced by direct environmental stimuli do not produce corresponding changes in the germ plasm. A gene which controls a particular somatic character is not correspondingly changed by an environment-induced change in that somatic character.

Genetic differences between individuals or generations may come about as a result of recombinations of genes, chromosomal aberrations, or changes in genes.

Recombinations of genes result from independent assortment during sexual reproduction. Two parents with the genotype *Cc* may produce offspring of three different genotypes, *CC*, *Cc*, or *cc*, one or two of which may be visibly different from the parents. Similarly, two parents with the genotype *AaBb* may produce offspring of nine different genotypes, *AABB*, *AABb*, *AAbb*, *AaBB*, *AaBb*, *Aabb*, *aaBB*, *aaBb*, or *aabb*, some of which may be visibly different from the parents. It is obvious, however, that the amount of genetic difference between parents and offspring that can come about by this process is limited by the number of heterozygous pairs of genes in the parents (if n represents the number of heterozygous pairs of genes in the two parents, these parents can produce offspring of 3^n different genotypes). The evolutionary change that has taken place in the past, as indicated by the fossil record, cannot have taken place by this process alone.

New or different combinations of genes may also result from chromosomal changes or aberrations. Chromosomal aberrations are changes in the number of chromosomes or changes in the structure of individual chromosomes. Chromosomal changes usually involve many genes, and hence a greater amount of genetic change can come about by this process than by independent assortment.

The chromosomes of an animal are normally present in pairs, one of each pair having come from each parent, or it may be said that the animal has two sets of chromosomes; this condition is spoken of as *diploid*, and the chromosome number is represented by the symbol $2n$ (n = the number of chromosomes in a set, 2 = the number of sets). Numerical changes in chromosomes are either an increase in the number of sets (*polyploidy*), or a change in the number of chromosomes in a particular set (*heteroploidy*). A polyploid condition in which there are three sets is called *triploid*, or $3n$; one in which there are four sets is called *tetraploid*, or $4n$. In heteroploidy one of a set may be missing ($2n-1$), or one set may contain an extra chromosome ($2n+1$); a $2n-1$ organism usually does not survive. Structural changes in the chromosomes may be *losses* (a section of a chromosome missing), *duplications* (a section of a chromosome present at its normal location and also present elsewhere), *translocations* (two non-homologous chromosomes exchanging parts), or *inversions* (a block of genes on a chromosome rotating through 180°). Chromosomal aberrations may often be detected by microscopic examination; inversions result in a peculiar behavior of the chromosomes at synapsis. Chromosomal changes of these various types have often been observed.

Genetic differences which come about by recombinations of genes or by chromosomal aberrations are always of a minor nature; judging from the fossil record, evolutionary changes have taken place which are greater in extent than can be accounted for by these processes alone. These more extensive changes in evolution must have come about by changes in genes, or mutations. The

term "mutation" as used here refers to a change in a gene[1], though some writers use the term to include also chromosomal aberrations; in actual practice it is sometimes difficult to determine whether a particular change in an organism has come about by a gene change or by a chromosomal change.

Mutations have been repeatedly detected in organisms, and quite a bit is known of their nature. A mutation may result in any of a number of changes in an organism—in physiology, and in the resultant visible structures, or in behavior. The change may be very marked, or it may be so slight that it can be detected only by special techniques. In its effect on the organism's ability to survive, a mutation may vary all the way from lethal (causing death) to increasing survival ability. Mutations apparently always occur in only one gene of an allelic pair at a time. They may occur in either body cells or germ cells; except in the case of animals which reproduce asexually, only mutations in the germ cells are of significance in evolution. Mutations may occur at any time in the life cycle of the organism. They occur "spontaneously." They occur under conditions favorable to the organism, and their causes are not well understood. They occur largely at random; when a mutation occurs in an organism it may be in almost any gene, and it may be in any of a number of different directions. The frequency with which genes mutate is very low; genes are generally very stable. Mutations resulting in marked effects on the organism usually occur less frequently than those resulting in only slight effects. The frequency of mutation varies with the gene (some genes mutate more frequently than others), with the allele produced (for example, the gene A may mutate more often to the gene a_1 than to the gene a_2), or with the race, strain, or species of organism involved. Certain environmental agents, such as X-rays and some temperature conditions, may influence the frequency of mutation; X-rays, for example, may increase the frequency some 150 times, but the mutations occurring are still at random. There is no known set of environmental conditions which will always produce only a specific mutation.

EVOLUTIONARY TREND-INFLUENCING FACTORS

A species or race which becomes altered in a particular direction by genetic or evolutionary changes does so either because of a restricted variety of mutations or recombinations, or as a result of differences in the survival of many random mutations. Continued evolutionary change in a given direction for a long period of time has taken place in the past, as indicated by the fossil record of many groups, but there is little genetic evidence that the variety of mutations is thus restricted. Such genetic evidence consists of the variation in the frequency of mutation of different genes, or of a given gene to different alleles, which in turn may be due to the inherent nature of the genes concerned or to the influence of other genes. Known mutations occur largely at random, hence evolution in a given direction must be principally a matter of certain mutations persisting in, and others disappearing from, a species population.

GENETIC DRIFT

Let us suppose that we have a species population in which all the genes of a particular allelic pair are the same, and we designate this gene type as A; this would mean that all individuals of this population are homozygous AA. Now, suppose that the mutation A to a occurs in one gene, with the result that the individual with this gene becomes heterozygous (Aa). We would then have a population consisting of one Aa individual and all the others AA. If the a gene in the Aa individual prevented that individual from mating, obviously the gene a would never get to the next generation (in a sexually reproducing animal),

[1]Following Dobzhansky, T., 1941, Genetics and the Origin of Species, Columbia University Press, New York, p. 22.

and there would be no evolution in this direction. If the *Aa* individual can mate, it must mate with an *AA* individual, and such a mating will result in either *AA* or *Aa* offspring or both.

These two parents might have almost any number of surviving offspring, depending on the species and on chance (let us assume that the *a* in the *Aa* individual is completely recessive, and hence the *Aa* individual has no greater survival or reproductive ability than the *AA* individuals). If they have no offspring surviving long enough to reproduce, the gene *a* is lost to the next generation. If they have one surviving offspring, there is a .50 chance of *a* being lost in the next generation; if there are two surviving offspring, there is a .25 chance of *a* being lost in the next generation; if there are *r* surviving offspring, there is a 2^{-r} chance of *a* being lost in the next generation. With the population remaining constant, generation after generation, the aggregate possibility of the gene *a* being lost in the next generation, considering varying numbers of surviving offspring, is .3679; the probability of its being in one individual in the next generation is .3679, in two individuals .1839, in three individuals .0613, etc.

As a result of this process, based as it is on chance, 36.79 percent on the average of all the new genes appearing in each generation by mutation are passed to the next generation unchanged in frequency, 36.79 percent are lost, and the rest are increased in frequency. It may be calculated that out of every 10,000 mutations, only 153 may be expected to survive 127 generations, assuming equal ability to survive and reproduce of *AA*, *Aa*, and *aa* individuals; these are the "lucky" genes, which increase in frequency instead of being lost.

It may be of interest at this point to note certain theoretical considerations concerning infinitely large populations in which the breeding is at random and in which there is no selection[2].

It can be shown mathematically that, in the absence of mutations, gene frequencies in such populations remain constant. This concept is usually known as the Hardy-Weinberg Law, and may be expressed by the equation

$$q^2AA + 2q(1\text{-}q)Aa + (1\text{-}q)^2aa = 1 \qquad (1)$$

where q = the frequency of the gene *A* and $(1\text{-}q)$ = the frequency of the gene *a*. This law means that if, for example, half the genes of a particular allele in the population are *A* and the other half are *a*, the ratio of the two genes will remain constant generation after generation.

It can also be shown that if the mutation *A* to *a* occurs in such a population, and if the reverse mutation *a* to *A* does not occur, then the gene *A* will eventually be eliminated from the population and all the individuals will become homozygous *aa*. The time in terms of the number of generations required for the gene *A* to be eliminated depends on the frequency with which the mutation *A* to *a* occurs.

It can further be shown that if both mutations, *A* to *a* and *a* to *A*, occur in such a population, then the frequencies of the genes *A* and *a* will reach a certain value and remain stable there. The frequencies at this stable point can be calculated; if we let *u* represent the frequency of the mutation *A* to *a* (which might, for example, be 1 in 100,000) and *v* represent the frequency of the mutation *a* to *A* (which, let us say, is 1 in 1,000,000), then the frequency of the gene *A* at the stable point is represented by *q* in the formula

$$q = \frac{v}{u + v} \qquad (2)$$

With the values of *u* and *v* mentioned, the value of *q* would be 1/11, that is, at the stable point one gene out of every eleven of this allele would be *A* and the other ten would be *a*.

These theoretical considerations suggest that in very large populations mutation (or "mutation pressure") would increase the frequency of occurrence of new

[2]Selection is discussed below, p. 325.

(mutant) genes in the population, and this would occur even if the new gene had an unfavorable effect on the organism, especially if the new gene were recessive. As a matter of fact, natural populations contain many recently mutated genes, even including lethals. This accumulation of new genes in a population results in what may be called *genetic variability*. If conditions change, the genotypes of a species population with high genetic variability may be reorganized and the species survive; populations with low genetic variability may be unable to effect reorganization of the genotype with changing conditions, and die out.

Populations of infinite size and with random breeding do not exist in nature; natural populations vary greatly in size, and those in which there is random breeding may be small. It can be shown mathematically that gene frequencies in small populations fluctuate at random, and some genes become fixed (homozygous in the entire population) while others become lost, purely by chance, and selection has very little effect. Evolution (changes in gene frequencies) is rapid until the genetic variability is lost, and selection is unimportant in influencing the direction of evolution. In large populations gene frequencies remain at or near a stable point, which is determined by mutation pressure (formula (2), above) and selection; evolution is slow or nil, and only severe selection is operative. The genetic variability is constant and high. Selection is of more importance in populations of intermediate size. There is abundant ecological, taxonomic, and genetic evidence that population size is often a very important factor influencing the fate of newly mutated genes and the genetic variability of populations, and hence in influencing evolutionary trends.

By population size is meant the size of the effective breeding population, which is not necessarily the same as the total number of adults. This value is influenced by many factors, including the extent of the movements of individuals during the mating or breeding season, territoriality and homing behavior, breeding behavior, and other factors. The biological significance of genetic drift in small populations lies in the fact that if a species is broken up into small isolated groups, these groups will become differentiated—by chance—even with similar environmental (and selectional) factors operating in the isolated groups.

It therefore appears that whether or not a new gene survives in a population and replaces the gene from which it mutated depends to a certain extent on chance, the chance being influenced by population size and the frequency with which the new gene is produced. However, in the preceding paragraphs we have spoken of *selection*; let us now consider the nature of this mechanism in influencing evolutionary trends.

SELECTION

The basic concept of selection as a trend-influencing mechanism in evolution is that the genetic characters of the better adapted individuals will become more frequent in the population in succeeding generations ("favored" by selection), while those of less adapted individuals will become less frequent. "Better adapted" means greater reproductive ability or greater survival ability, or both. Greater reproductive ability means higher fecundity or greater mating success; greater survival ability involves greater ability to resist such environmental hazards as enemies, competition, and other environmental factors that are not optimum. The trend of evolution, if influenced by selection, is thus in the direction of adaptation.

The effect of selection is proportional to its intensity, or what for purposes of mathematical treatment may be termed the selection coefficient. For example, if s is the coeffiicient of selection favoring the gene A over its recessive allele a (that is, favoring AA or Aa over aa), then for every offspring produced by the dominants the recessives produce 1-s offspring, on the average. If the frequencies are A and a are .5 in one generation, and the value of s is .01, the value of q (fre-

quency of the gene *A*) in the next generation would be .5012; with an *s* value of 1 (which would mean that the gene concerned is a recessive lethal) the value of *q* in the next generation would be .67.

The effect of selection varies with the frequency of the genes concerned. With a constant selection coefficient, the effect of selection decreases as the frequency of the favored gene increases. For example, starting with *q* (the frequency of the favored gene *A*) equal to .5 and a selection coefficient of 1 (a recessive lethal), the value of *q* in the second generation would be .67 (an increase of .17), in the third generation .75 (an increase of .08), in the fourth generation .80 (an increase of .05), and so on; in the *n*th generation *q* would be $\frac{n}{n+1}$. The increase each generation in the frequency of the dominant allele favored by selection may be calculated by the formula

$$q_2 = \frac{q_1}{1-s(1-q_1)^2} \tag{3}$$

The above discussion of the effect of the intensity of selection (*s*) is based on the assumption that there are no mutations; mutations alter the picture slightly. With mutation away from a favored allele, an equilibrium will be established; for example, with *A* mutating to the less favorable allele *a* with a frequency of *u*, the equilibrium frequency of *a* (q_a) may be calculated by the formula

$$q_a = \sqrt{\frac{u}{s}} \quad (s = \text{the selection coefficient}) \tag{4}$$

Where $s=1$ (a recessive lethal) the formula becomes

$$q_a = \sqrt{u} \tag{5}$$

If the selection coefficient is high, mutation has little effect, and the frequency of the gene selected against remains low. If the selection coefficient is low, the mutation frequency is more important in determining gene frequencies, which tend to remain at equilibrium at a point determined by the mutation rates to and from the favored allele (formula (2), above).

In cases where selection favors heterozygotes over homozygotes—and such cases are known—the proportions of heterozygotes and homozygotes reach a balance; this situtation is sometimes referred to as a *balanced polymorphism.*

Selection may also play a role in the evolution of non-adaptive characters. Neutral or even slightly deleterious traits may be favored by selection if they happen to be connected with favorable ones; selection acts on the *over-all* adaptive value of the genotype. Further, a seemingly neutral trait may be only one effect of a gene, the other effects of which may be favored by selection. Due to the relationships between developing parts of the organism, a change in one organ may result in changes in developmentally related organs.

As a result of mutation, traits may appear which are adaptively neutral in one environment, but which may be of adaptive value in another environment; this phenomenon is sometimes termed *preadaptation.* Evolution by selection occurs because some genotypes happen to have adaptive value in environments encountered by the species. Mutations do not occur when needed, but if they happen to be present when needed, selection will favor them.

Evolution by selection may be centripetal, centrifugal, or linear. In centripetal evolution, selection favors the average condition, and variants tend to be eliminated; there is an increase in specialization, with a reduction in the variation in the species. In centrifugal evolution the variants are more adaptive; this process might be compared to an expanding ring, and if the variants become isolated further divergence and speciation may occur. Linear evolution occurs with a slow shift in environmental conditions (selection factors), with the average of the population shifting. With a constant shift in selection factors (orthoselection), the evolution is linear or orthogenetic.

ISOLATION

Natural populations of organisms exhibit discontinuous variation. The world fauna and flora consist of many different populations which differ in non-integrading characters, that is, all the individuals of one population are different from all the individuals of the other populations. These population units are called species or races, the term used depending somewhat on the populations; they usually differ in many genes, and often also in chromosome structure. The maintenance of this discontinuous variation depends upon the different populations being prevented from interbreeding. The interbreeding of races or species (where such interbreeding is possible) results in a breakdown of the racial or specific differences, though the individual genes remain unchanged. The discontinuous variation of races and species can come about only when population groups are *isolated*, that is, when they are prevented from interbreeding.

The mechanisms which prevent the interbreeding of groups of individuals, and thus bring about isolation, are very diverse. Groups may be isolated by geographic barriers (*geographic isolation*), and hence never meet. Two groups may occur in different ecological situations in the same general region, yet never come together during the breeding season (*ecological isolation*). Two groups may occur in the same habitat and interbreeding may not occur because the breeding periods of the two groups are at different times (*seasonal* or *temporal isolation*). Two groups may occur together and not interbreed because of a lack of mutual attraction of the two sexes (*sexual isolation*), or copulation may be difficult or impossible due to the physical incompatibility of the reproductive organs (*mechanical isolation*). In some cases mating between two groups may occur, with no resulting offspring due to a failure of fertilization or development, or with the offspring sterile.

One effect of isolation is that the isolated groups evolve in different directions in certain respects. As we have seen, the mutations occurring in a population are largely random in nature, and whether a particular new gene that appears in a population persists or is lost depends on chance and selection. In some cases, particularly in small populations, selection has relatively little effect. Therefore, because of the random nature of mutations, and the role of chance in determining the fate of new genes, one would expect that in general the new genes which appear and persist in isolated populations would be different, and hence the isolated populations would evolve in different directions. The longer the period of isolation, the more the isolated populations would diverge. In cases where selection is particularly severe, and similar in the isolated populations, these isolated populations would probably exhibit similar evolutionary trends.

The data of geographic distribution furnish considerable evidence to support the role of isolation in species formation. A species' nearest relative is usually to be found in an adjacent area separated by a barrier; exceptions may be due to some type of isolation other than geographic, or the related forms may have come together by migration subsequent to their divergence. The degree of isolation of any given area for different species varies inversely with the vagility (ability to extend the range) of the species. A species of high vagility may be represented in a given area by a single form, while another, of low vagility, may be split into a number of geographic races in the same area. The amount of endemism (a species is endemic to an area when it occurs there and nowhere else) in various groups in a given area usually varies inversely with the vagility of the groups. The amount of differentiation of a group in an isolated area is influenced by the duration of its isolation, population size, and selection. Long-continued isolation of a group may be accompanied by its evolving into ecological types, similar in adaptations but different taxonomically, from species in similar habitats elsewhere. Similar areas, such as deserts, mountains, and grasslands, in different

parts of the world are in general occupied by different species. Each species is usually restricted to particular geographic areas, and does not occupy all the geographic areas in the world where conditions are suitable for its existence.

RATES OF EVOLUTION

The rate of evolution may be defined as the amount of change per unit of time. It may be measured in terms of the amount of morphological change, as evidenced by the taxonomic units applied to the different stages of the evolving line. Data on evolutionary rates are largely from the field of paleontology.

Many variations occur in rates of evolution. In a given line, the rate may vary in different geological periods. Characters in a given evolving line which are developmentally correlated may evolve at similar rates, but characters not developmentally correlated may evolve at different rates. In some cases, at least for short periods, a given character may evolve at a constant rate. It would seem that evolution should be faster in forms with more generations per unit of time, but the fossil record shows no correlation between rate of evolution and generation length (compare the fossil records of flies and elephants during the Cenozoic).

Different lines within a group such as a class, order, or family, may evolve at different rates; some (*tachytelic*) may have a high rate of evolution, while others (*bradytelic*) may have a low rate. Tachytely is usually of short duration, and often ushers in a new major group; then the rate is more moderate (*horotelic*).

The fossil record contains many examples of bradytelic lines—groups which have evolved relatively little over long periods, for example, horseshoe crabs (Triassic to present), opossums (late Cretaceous to present), and many molluscan genera (Carboniferous to present). Bradytely is likely in groups with continuously high populations and stable environmental conditions, even with normal mutation rates. Such forms usually have a high range of tolerance, and are not overspecialized.

SUMMARY

By the process of organic evolution present-day organisms have descended, with changes, from the organisms of the past. Some of the characteristics of the evolutionary process can be inferred from the data of paleontology and geographic distribution. The changes which occur in evolution involve genetic changes in individuals and populations, and come about largely through mutation. The direction in which a population evolves may be influenced by its inherent mutability, the population size, selection, and isolating mechanisms. Evidence of the nature and mechanism of evolution is to be found in the data of paleontology, observations of mutations in experimental animals and plants, the results of experimental breeding, studies of the genetics of natural populations, and the data of geographic distribution.

Cells and Societies. *J. T. Bonner.* Princeton Univ. Press, Princeton, New Jersey. 1955. 234 pp. $4.50.

There is a sameness of living things whether they be cells or societies. It is concerned with certain activities that they all have in common, notably those of food-getting, reproduction, and coordination or integration.

Professor Bonner develops this theme in twenty-three chapters that are short, lucid, and imaginative in style. He first describes the behavior patterns exhibited by certain mammalian societies such as howling monkeys, fur seals, red deer, and beavers. Insectan societies are next described, followed by colonial animals and single-celled forms. The second half of the book is concerned with short generalized essays on plants and other biological activities of animals.

This book should prove to be interesting and informative reading to the layman and the student with a "non-biological major."

JAMES G. HAUB

EFFECT OF ADRENOCORTICAL EXTRACT AND RELATIVELY SMALL DOSES OF SESAME OIL ON THE OXYGEN CONSUMPTION OF GOLDFISH[1]

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Sesame oil has been shown (Pollia, 1937; Bruce and Tobin, 1940; Clausen, 1940; Spurr and Kochakian, 1940; Crafts, 1942; Calhoon and Angerer, 1950a) to produce a variety of effects in animals, despite the fact that it and other carrying agents have been considered more or less biologically inert (Brown, Wilder and Schwartz, 1944). It has been reported (Calhoon and Angerer, 1950a) that heavy doses of sesame oil (0.0036 ml./gm. body weight injected 3×/week for 8 to 10 weeks inclusive) decrease the rate of oxygen consumption of goldfish by *ca.* 37 percent when compared with controls, and that adrenocortical extract (ACE) injected simultaneously with sesame oil "protects" these animals from this effect. The total dosage of sesame oil previously administered (Calhoon and Angerer, 1950a) was relatively large, being equivalent to 252 ml./70 kg. However, it is comparable in magnitude to dosages previously reported (Pollia, 1937; Bruce and Tobin, 1940; Clausen, 1940; Spurr and Kochakian, 1940; Crafts, 1942). This study was undertaken for the following reasons: to determine (1) whether a smaller (1/20) volume, *i. e.*, a more clinical dose, of sesame oil than hitherto reported produces a metabolic disturbance; (2) whether ACE antagonizes the foregoing disturbance; and (3) whether the effect of ACE on the metabolism of normal poikilotherms can elucidate the confused literature existing for homoiotherms.

METHODS

The common goldfish, *Carassius auratus*, was used as the experimental animal in the hope that a poikilothermic vertebrate might produce reactions otherwise obscured by the more efficient regulatory mechanisms, *e.g.*, temperature and endocrine, existing in homoiotherms. The fish were obtained, maintained and rendered in a "basal" state as previously described (Calhoon and Angerer, 1950a).

All determinations of oxygen consumption were made by direct reading volumeters (Calhoon and Angerer, 1953). This method has the same order of experimental accuracy as obtained by the Winkler method (Calhoon and Angerer, 1950a; Calhoon, 1953). Determinations of oxygen consumption were made at 15 min. intervals for a period of at least 1 hr. The rate of oxygen consumption was calculated on the basis of ml./gm. (wet) of fish/hr. at 30.00 ± 0.01°C. The extremely small injections of sesame oil and of ACE were accomplished by means of a micro-syringe constructed according to the general specifications described by Woodrow (1949).

RESULTS

Sesame oil. Determinations on 12 different "normal" fish gave a mean value of 0.296 ± 0.115 (standard deviation) ml. O_2 consumed/gm./hr., at 30°C (zero week, line 1, table 1). These fish were then injected intramuscularly with 0.00018 ml. of sesame oil/gm. of body weight 3×/week during 7 consecutive weeks. In table 1 there are found the various mean values for oxygen uptake (column 4)

[1]Aided by grants from the Comly-Coleman Research Fund, College of Medicine and from the Graduate School, The Ohio State University.

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with their respective standard deviations (column 5) which were obtained at the end of the week indicated (column 1) after the initial injection. At the close of the second week there was an apparent transient increase of *ca.* 9 percent in the rate of oxygen consumption. This was immediately followed by a sharp decrease until the end of the seventh week when the value was *ca.* 37 percent below the initial (zero time) value.

Sesame oil plus ACE. The mean oxygen consumption (control) was determined for 12 different normal goldfish, and it gave 0.397 ± 0.130 ml. of O_2 consumed/gm./hr. These fish were then injected intramuscularly 3×/week for 7 consecutive weeks with 0.00018 ml. of sesame oil plus 0.00036 ml. of Upjohn's Adrenal Cortical Extract/gm. of body weight. The rates of oxygen consumption of individual fish were determined at the close of each of 7 consecutive weeks. The means of these data are presented in paired form with their respective controls (sesame oil injected fish) in table 1. Although the initial rates of oxygen consumption of the 2 groups of animals gave different values (*cf.* 0.296 and 0.397 ml. O_2 consumed/gm./hr.) they were not significantly different ($P = 0.08$, Student's method). Analysis of these data (table 1) shows that at the end of the second week the oxygen consumption had decreased *ca.* 35 percent compared with the initial week, and remained near this value for the next 5 wk. A comparison of the mean oxygen uptake of the ACE-oil-treated group with the oil-treated (control) group for any given week (table 1, Column 8) indicates significant decreases at the end of 2, 3 and 6 wk. of *ca.* 52 percent ($P < 0.01$), 44 percent ($P < 0.01$) and 30 percent ($P < 0.02$), respectively.

Sesame oil plus ACE on body weight (table 2). The rates of gain in mean body weight of these 2 groups of animals fail to exhibit the linearity which was obtained for a similar experiment involving larger volumes of sesame oil (Calhoon and Angerer, 1950a). At the end of 7 wk. as compared with zero week the mean increase in body weight of fish given ACE plus sesame oil (table 2) is *ca.* 13 percent while in the same interval, the mean increase for fish given sesame oil alone is *ca.* 11 percent (table 2). The difference between these mean values at the end of the seventh week is not statistically significant ($P > 0.20$).

DISCUSSION

The effects of small doses of sesame oil on the total oxygen consumption of goldfish (table 1) is open to the same interpretation as was suggested for the effects of large doses, *i. e.*, they cause liver damage (Calhoon and Angerer, 1950a). There was, as expected, a time difference: "large" doses (0.0036 ml./gm.) of sesame oil produced *ca.* 30 percent metabolic depression at the end of 1 wk. (Calhoon and Angerer, 1950a), while "small" doses (0.00018 ml./gm.) produced the same degree of depression, but only after 5 to 6 wk. This may be explained as due to the cumulative effect of the oil (Brown, Wilder and Schwartz, 1944). Indeed, the amount of oil accumulating as a result of "small" doses at the end of 5 to 6 wk., assuming 50 percent loss (Brown, Wilder and Schwartz, 1944), is the same as for 1 "large" dose given at the end of 1 wk. (Calhoon and Angerer, 1950a). Thus whether the oil is given in small doses over a long period of time, or in large doses over a short period of time, the same critical level is reached, producing the same degree of depression (*ca.* 25–35%) in total oxygen consumption.

While "small" doses of sesame oil have little effect on the total oxygen consumption of goldfish for the first 3 wk, the same quantity of sesame oil plus ACE (table 1) depresses the oxygen consumption of these fish. The difference between these 2 sets of mean values at the end of 2 and 3 wk. is *ca.* −52 percent ($P < 0.01$) and −44 percent ($P < 0.01$), respectively, and must be interpreted as due to the action of ACE on oxygen consumption. Investigations with frogs show relatively the same degree of depression in oxygen consumption when ACE is given alone (Calhoon and Angerer, 1955). These results, indicating a decrease in oxygen

TABLE 1

Sesame oil plus ACE on total oxygen consumption

Week	Treatment	No. of Fish	Mean ml./O_2/gm./hr.	Std. Dev.	Weekly Comparison with *Own zero week* % Diff.	Weekly Comparison with *Own zero week* P-Value**	Weekly Comparison of Both Groups*** *Using Oils as Control* % Diff.	Weekly Comparison of Both Groups*** *Using Oils as Control* P-Value**
0	oil	12	0.296	0.115	....	....		
	" +ACE	12	0.397	0.130	....	....	+34.1	0.08
1	"	12	0.297	0.062	0.3	....		
	" +ACE	12	0.355	0.087	−10.6	0.40	−14.5	0.20
2	"	10	0.322	0.076	+ 8.8	0.60		
	" +ACE	10	0.257	0.044	−35.3	0.01	−51.9	0.01
3	"	10	0.298	0.043	0.1	....		
	" +ACE	10	0.268	0.052	−32.5	0.01	−43.9	0.01
4	"	10	0.233	0.106	−21.3	0.20		
	" +ACE	10	0.312	0.090	−21.4	0.10	−.9.9	0.60
5	"	10	0.234	0.069	−21.0	0.20		
	" +ACE	4*	0.253	0.079	−36.3	0.10	−35.0	0.07
6	"	10	0.245	0.055	−17.2	0.20		
	" +ACE	10	0.273	0.067	−31.2	0.02	−29.8	0.02
7	"	10	0.186	0.032	−37.2	0.01		
	" +ACE	10	0.280	0.094	−29.5	0.01	− 3.0	0.90

*Only 4 fish run this day because of unavoidable circumstances.

**Probability of there being no difference between experiment and control values.

***Since there is no statistically significant difference between the means of the 2 groups on the initial week, the apparent mean difference (0.101) was added to the difference between the 2 points at each successive week's analysis.

consumption of normal fish treated with ACE, tend to agree with those in the literature on rabbits (Marine and Baumann, 1922) and dogs (Franke, 1932) but to disagree with those which give either an increase for dogs (Koehlar and Hastings, 1929; Hoffman and Talesnick, 1948) or no change for dogs (Harrop, Pfiffner, Weinstein and Swingle, 1931; Missiuro, Dill and Edwards, 1938), cats (Harróp, Pfiffner, Weinstein and Swingle, 1931) and humans (Hitchcock, Grubbs, and Hartman, 1938).

It is suggested that the metabolic depression observed in these cold blooded animals is due to a direct action of ACE on tissue metabolism. This is in line with previous reports to the effect that various adrenocortical fractions depress the QO_2 values of brain and liver slices (Tipton, 1939; Gordon, Bentinck and Eisenberg, 1951), the oxidation of glucose by tissues (Long, Katzin, and Fry, 1940; Evans, 1941), the activities of various dehydrogenases in brain slices (Gordon, Bentinck and Eisenberg, 1951) and the actions of various other enzymes (D amino-acid oxidase, tyrosinase, urease, transaminase and ascorbic acid oxidase) in kidney slices (Umbreit, 1951; Hayano, and Dorfman, 1951). However, it is possible that the observed depression in oxygen consumption of goldfish, as well as of frogs (Calhoon and Angerer, 1955), may involve a dysfunction of the pituitary, since a pituitary-adrenal cortex relationship is known to exist at least for the frog (Atwell, 1935, 1937). This aspect of the problem must await further development.

It is suggested that the difference observed between this and a previous report (Calhoon and Angerer, 1950a) is interpretable on the basis that in the latter study, sesame oil *per se* produced a "stress"; while this metabolic "stress" was nullified in the presence of adrenocortical extract. However, in the present report, during the first 2 wk. no metabolic disturbance was produced by the injected sesame oil and the ACE was free to produce its inhibitory effect on total oxygen consumption. It is felt that this is especially true, since it is anticipated that were the present study carried on for a longer time the mean difference between the two sets of data for oxygen uptake (column 4, table 1) would decrease to zero and subsequently diverge in a manner similar to that represented by the curves previously published (Calhoon and Angerer, 1950a) on the effects of large doses of sesame oil alone, and together with ACE.

There is a progressive increase with time in body weight for the sesame injected fish. At the end of 7 wk. *ca.* 11 percent increase occurs when compared with zero week. When ACE is injected simultaneously with oil the body weights tend to oscillate around the respective controls. There is no trend to show any acceleratory effect on body weight nor any degree of linearity between dosage and time as was observed for "large" doses of sesame oil (Calhoon and Angerer, 1950a).

SUMMARY

1. Goldfish injected intramuscularly with 0.00018 ml. of sesame oil gm. body weight 3×/week for 7 wk. show relatively no change in oxygen consumption during the first 2 or 3 weeks (table 1). Thereafter, a progressive decrement is attained. The data indicate a continuing decrease were the experiments carried on for a longer time.

2. Fish injected with 0.00018 ml. of sesame oil simultaneously with 0.00036 ml. of ACE gm. body weight 3× week for 7 wk. give *ca.* −35 percent in mean oxygen consumption at the end of 2 weeks with respect to the controls. This value tends to be maintained thereafter for the duration of the experiment.

3. When ACE and oil are injected simultaneously and the body weights are determined on a weekly basis, their mean values tend to oscillate about the respective controls. This is unlike the effect of "large" doses of ACE and sesame oil.

TABLE 2

Sesame oil plus ACE on mean body weight

Week	Treatment	No. of Fish	Mean Body Wt.	Std. Dev.	Weekly Comparison with *Own zero week* % Diff.	Weekly Comparison with *Own zero week* P-Value**	Weekly Comparison of Both Groups *Using Oils as Control* % Diff.	Weekly Comparison of Both Groups *Using Oils as Control* P-Value**
0	oil	12	6.3	2.8	.....	....		
	" +ACE	12	4.8	1.0	.....	....	−23.8	0.10
1	"	12	6.6	2.7	+ 4.8	0.80		
	" +ACE	12	4.7	1.0	− 2.1	0.80	−28.8	0.02
2	"	10	6.4	1.8	+ 1.6	0.30		
	" +ACE	10	5.2	1.0	+ 8.3	0.30	−18.8	0.10
3	"	10	6.4	1.7	+ 1.6	0.90		
	" +ACE	10	5.2	1.1	+ 8.3	0.40	−18.8	0.10
4	"	10	7.5	1.8	+19.0	0.20		
	" +ACE	10	5.0	1.2	+ 4.2	0.60	−33.3	0.01
5	"	10	6.9	1.6	+ 9.5	0.50		
	" +ACE	4*	5.9	0.9	+22.9	0.05	−14.5	0.10
6	"	10	6.7	1.8	+ 6.3	0.60		
	" +ACE	10	5.2	1.2	+ 8.3	0.40	−22.4	0.05
7	"	10	7.0	1.8	+11.1	0.40		
	" +ACE	10	5.4	1.1	+12.5	0.20	−22.9	0.02

*Only 4 fish run this day because of unavoidable circumstances.
**Probability of there being no difference between experiments and control values.

4. The conclusions suggested from these results and from their comparison with a former study (Calhoon and Angerer, 1950a) using "large" doses of sesame oil are: (a) above a critical dosage, either single or cumulative, sesame oil depresses total oxygen consumption of goldfish; (b) ACE given simultaneously with sesame oil antagonizes (inhibits) the metabolic depressant action induced by the oil alone; (c) when given in excess of this amount it causes *ca.* −37 percent in total oxygen consumption with respect to the controls, which is interpreted as due to the action of ACE *per se*.

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STUDIES ON BACTERIAL INTERACTION: I. THE EFFECT OF TRYPSIN ON THE CELLULASE ACTIVITY OF *CELLULOMONAS FLAVIGENA*[1]

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Since cellulose is the most abundant of all carbohydrates and since only a relatively small amount of that produced annually is utilized by man, one of the most attractive possibilities in the realm of microbiology is the conversion of cellulose to more valuable compounds by biological means. Ever since the early definitive studies of Omelianski (1902), efforts have been directed toward the realization of this possibility. Many bacteria have been shown to possess enzymes capable of digesting cellulose, but purification of cultures has typically resulted in a diminution of cellulose activity. Several explanations have been advanced to account for this reduction in activity, many being based on the idea of beneficiation of the medium through the production by the "contaminants" of vitamins (Cowles and Rettger, 1931) or other required substances (Burroughs *et al.*, 1950). It is the purpose of the present paper to suggest that increased cellulase activity so often noted in mixed bacterial cultures may result from the direct action of enzymes on the cellulase synthesizing organisms.

MATERIALS AND METHODS

The organism used in these studies, *Cellulomonas flavigena*, was obtained from Dr. E. T. Reese of the Quartermaster General Laboratories, in Philadelphia, Pa. This species was isolated and its cellulase activity demonstrated by members of the staff of that laboratory (Reese, 1948, Siu, 1950). The stock culture was propagated in triplicate on sucrose agar containing peptone and yeast extract. These cultures were stored at 10° C after growth was apparent, transfers being made at 60 day intervals.

The cells used in the investigation were cultured on sucrose or cellulose agar. Good growth was obtained on the former in 3 days; growth on cellulose agar was less rapid, being apparent only after 20 days. In either case, the cells were suspended in buffered distilled water (pH 7.0) and were washed 3 times. After 24 hr. incubation at 10° C, the suspension was so diluted that a 1: 20 dilution transmitted 60 percent of the incident light in an Evelyn photoelectric colorimeter when transmission through distilled water was taken as 100 percent.

Trypsin was used in this preliminary study because it was readily available and because it has a fairly broad action spectrum. Trypsinized cells were obtained by incubating washed cells in a 1: 20,000 solution of trypsin at pH 7.0. Following 30 min. incubation in a 37° C water bath, the trypsinized cells were washed 3 times to remove the residual trypsin prior to use. Cells not treated with trypsin were subjected to the same incubation and washing before use.

Ground cells were obtained by triturating washed, stored cells in a mortar. Alumina was used to enhance abrasion. When the resultant slurry was trypsinized, boiled trypsin was added to the control preparation, it being impractical to attempt to remove the residual trypsin by washing.

The activity of the various preparations was measured by manometric techniques described by Umbreit *et al.* (1949). The substrates used were carbo-

[1]Portions of this paper were included in a dissertation presented by the senior author in partial fulfillment of requirements for the degree Doctor of Philosophy, Department of Bacteriology, The Ohio State University.

[2]Paper No. 551 from the Department of Botany, and Plant Pathology.

hydrates having glucose and/or its isomers as the basic unit. All were used in M/50 concentration calculated as glucose equivalents.

EXPERIMENTAL RESULTS

C. flavigena, cultured on sucrose agar, was tested for its capacity to oxidize glucose, sucrose, cellobiose, cellulose, starch and inulin, both before and after trypsinization. The oxidation of starch and inulin followed the pattern obtained with cellulose; the balance of the substrates followed the oxidative pattern established for glucose. Since the oxidation of these substrates thus fell into two groups, the data obtained with glucose and cellulose are reported as representative. Figure 1 summarizes the data obtained in this part of the investigation.

From the curves presented in figure 1, it is obvious that cells of *C. flavigena* which had been cultured on sucrose agar were able to oxidize glucose but unable to oxidize cellulose prior to trypsin treatment. After exposure to trypsin the cells oxidized glucose more rapidly. Oxygen uptake by trypsinized cells in the presence

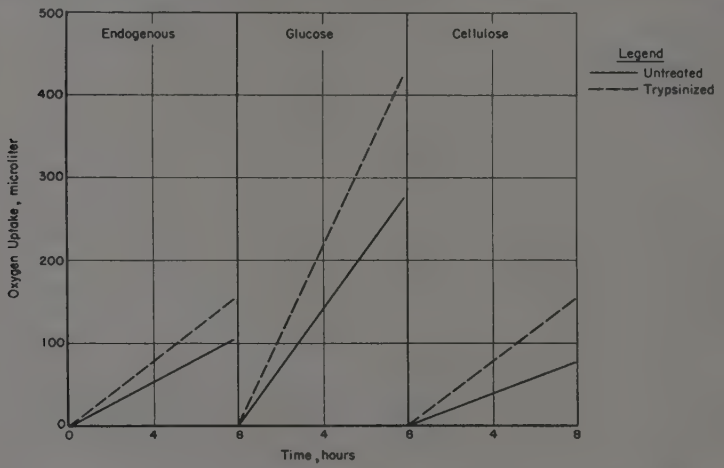

FIGURE 1. Oxygen uptake by *C. flavigena* (cultured on sucrose) before and after trypsinization.

of cellulose was greater than oxygen uptake by untreated cells; however, endogenous utilization of oxygen was also increased in trypsinized cells, and so the increased oxygen uptake in the presence of cellulose was not significant.

The data obtained when "cellulose-grown" cells were tested for their capacity to oxidize the test substrates are presented in figure 2.

From the data shown in figure 2, it is apparent that "cellulose-grown" cells oxidized glucose and to a lesser extent cellulose. Trypsinization of these cells increased the rate of oxidation of glucose but not that of cellulose. The endogenous oxygen utilization by these cells was not affected by treatment with trypsin.

It was hoped that treatment of *C. flavigena* with trypsin would bring about an increase in the rate of cellulose utilization, since it has been claimed that cellulase might be found on, or in the cell membrane (Rahn and Leet, 1949). Since trypsin neither unmasked cellulase on the "sucrose-grown" cells nor removed cellulase from "cellulose-grown" cells, it was supposed that cellulase was protected by virtue of its position or by some other as yet unelucidated mechanism. This supposition was tested by subjecting "sucrose-grown" cells to the action of trypsin after they had been ground in a mortar. The preparations were tested for their ability

to oxidize the substrates previously mentioned. The data obtained are presented in figure 3.

It is evident that the endogenous rate of oxygen utilization was high with both of these preparations. The shape of the curves obtained indicates that growth was

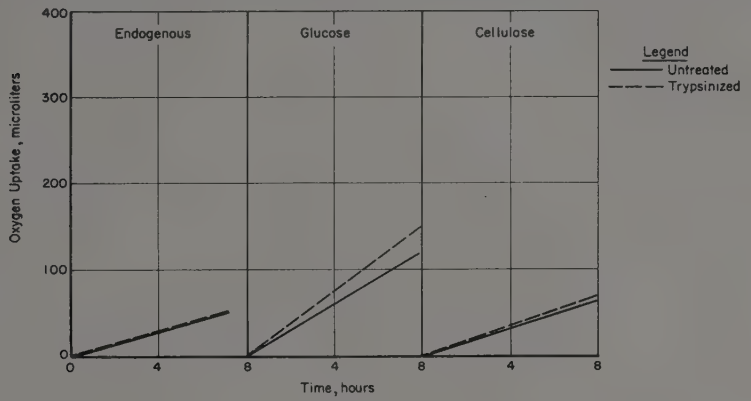

FIGURE 2. Oxygen uptake by *C. flavigena* (cultured on cellulose) before and after trypsinization.

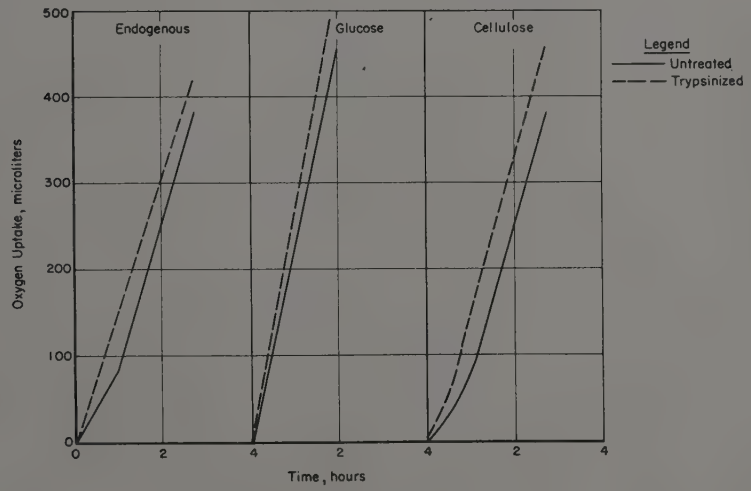

FIGURE 3. Oxygen uptake by *C. flavigena* (cultured on sucrose) before and after trypsinization.

probably taking place in the Warburg flasks. The most interesting observation is that the slurry from "sucrose-grown" cells was capable of oxidizing cellulose after being ground and subjected to the action of trypsin. Similar results were obtained when "cellulose-grown" cells were treated in like manner.

DISCUSSION

In attempting to relate the data obtained to events as they may occur in mixtures of organisms, it is necessary to make a few assumptions. Although many mixtures of microorganisms are characterized by a fairly constant rate of

reproduction, increases in numbers are often transitory. It should be noted that although we are accustomed to speaking of the "protein-sparing action of carbohydrates", this phenomenon may not be too common. In complex mixtures of organisms and substrates, of which sewage is an example, both carbohydrates and proteins are digested readily. It is therefore reasonable to assume that proteolytic enzymes are liberated into the same medium in which those capable of hydrolyzing carbohydrates are liberated. Peltier and Schroeder (1949) have shown this to be the case with *Bacillus subtilis* and have concluded that production of amylase without concurrent release of proteolytic enzymes by strains of this species would be a remarkable event.

In the light of available information, it is possible to postulate that in mixed bacterial cultures at least some synergistic reactions are brought about through the direct action of enzymes on bacterial cells. Although there is no reason to believe that trypsin is produced by bacteria, there are similar proteolytic enzymes that are known to be synthesized by these organisms.

Enzymes similar to trypsin might be considered to have a dual action; altering the permeability of living cells, and releasing enzymes from disrupted cells. The enzymes released from cellular debris would increase the concentration of diffusable substances which could be utilized more rapidly by viable cells exposed to the action of proteolytic enzymes. The data presented indicate that trypsin probably increased the permeability of viable cells as well as liberating cellulase from cellular debris.

In natural mixtures of microorganisms, these events might lead to a cycle in which proteolytic enzymes would increase certain activities of viable cells thus enhancing reproduction. As the cells multiplied, other factors might intervene and reduce the numbers of living cells. The dead and dying cells would then be subject to digestion during which process enzymes would be released. These enzymes in turn would act, increasing the concentration of diffusable substances and completing the cycle. The result of such a course of events would be increased activity of all cells with a net increase in utilization of a particular substance.

While the above discussion is oversimplified and admittedly speculative, it does suggest a somewhat different approach for further study of microbial interactions.

SUMMARY

Cellulomonas flavigena is capable of oxidizing cellulose when the cells are cultured on cellulose-containing medium, but not when the cells are cultured on sucrose-containing medium. Treatment of both preparations with trypsin increases the rate of glucose oxidation but has no effect on the rate of cellulose oxidation. When either preparation is ground prior to trypsin treatment, its capacity to oxidize cellulose is enhanced. An attempt is made to relate the data obtained to events as they might occur in mixed culture.

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THE EXTRACTION OF THE PIGMENT FROM TOURACO FEATHERS BY SOAP SOLUTION

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We wish to record an unusual instance of the extraction of the pigment from a bird's feathers during washing. In 1952, the attendants at the zoo of the Zoological Society of Cincinnati noted that when a two year old, female touraco (an African bird belonging to the family Musophagidae) was given a sponge bath, the bath water was tinged with red. It was suspected that either the bird's feathers had been dyed by an unscrupulous dealer or some contaminant or additive in the water had served to extract the pigment.

The red color in the quill feathers of the touraco is due to turacin, which was shown by Fischer and Hilger (1923, 1924) to be the copper complex of uroporphyrin. Although these investigators believed the compound to be a salt of uroporphyrin I, more recent work (Rimington, 1939; Nicholas and Rimington, 1951) indicates that turacin samples obtained from eleven different species of touracos are all derived from uroporphyrin III; the main porphyrin is accompanied by another porphyrin which, like uroporphyrins I and III, contains 8 carboxyl residues. Eight *free* carboxyl groups are present in the uroporphyrins, and hence it is not surprising to find that turacin is soluble in very weakly alkaline solutions, *e. g.* 1 percent aqueous amonia, and may be extracted from feathers in this manner.

The soap employed in washing the bird in question was a potassium soap said to have a pH less than 9 and containing the antiseptic hexachlorophene. When an acidic soap was substituted, no further extraction of pigment was noted. When touracos are bathed in plain water, a coloration of the bath water is also sometimes noted (Church, 1869), and has been ascribed to the alkalinity of the feces which may contaminate the water with the trace of alkali needed to effect turacin solubilization (Fischer and Hilger, 1924). It is fortunate, from the animal keeper's standpoint, that uroporphyrin pigments are rare and have been found in no other vertebrates.

This problem was brought to the author's attention by Mr. N. S. Hastings of the Zoological Society of Cincinnati.

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RESPONSES OF THE OAT COLEORHIZA TO VARIOUS TREATMENTS IN CULTURE[1]

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The coleorhiza or root sheath of the grasses has received little attention from plant physiologists and experimental morphologists. Nevertheless it is of importance in the germination of the oat seed and is interesting in its responses to certain factors in its immediate environment.

In a number of cultures of excised oat embryos, extreme coleorhizal elongations were observed. Such elongations occurred regularly in liquid media. However, the coleorhizae of oat embryos cultured on the surface of nutrient agar elongated only slightly. Instead they produced numerous epidermal hairs which appeared to be identical to root hairs. These phenomena have been reported by other investigators. Nishimura (1922) and Howarth (1927) consider that such coleorhizal hairs serve to supply water to the embryo during germination. Dietrich (1924) discovered that greatly lengthened coleorhizae were developed in subsurface cultures of excised oat embryos. LaRue (1936) observed that immature, excised embryos of certain grasses formed coleorhizal hairs *in vitro*.

Normally the coleorhiza is the first part of the oat embryo to break through the seed coat during germination. At this time it further elongates and forces itself through the husk or lemma surrounding the seed. When this is accomplished coleorhizal elongation ceases. Hairs then are often formed on the parts of the coleorhiza exposed to air. The following account deals with oat coleorhizae subjected to various treatments.

MATERIAL AND METHODS

Seeds of oats, *Avena sativa* L. variety *Andrew*, were obtained from the North Dakota Agricultural Experiment Station. Some of these were planted to provide embryos of various stages of development. Other seeds were cultured in sterile water or on moist filter paper. A simple apparatus was used in the latter cultures to obtain various dilutions of air with nitrogen gas. Excised embryos, of different ages, were cultured in White's solution (White 1943). All seeds were sterilized in a 10 percent solution of *Clorox* for ten minutes prior to culture.

RESULTS

Coleorhizal growth in liquid culture. Coleorhizal elongations were noted in excised embryos cultured for a week in White's solution but not in those cultured in sterile water alone. Coleorhizal elongations also occurred when intact seeds were placed either in White's solution or in sterile distilled water. Because of the ease with which they could be cultured in sterile water, whole seeds rather than excised embryos were used in the experiment reported in table 1. This experiment was performed in order to discover whether or not coleorhizal elongations were due solely to the presence of water. The effect of indole-3-acetic acid on coleorhizal elongation was also tested.

The effect of various factors on the growth of oat coleorhizae. As a result of experimentation, the data of which appear in table 1, some factors affecting coleorhizal behavior have been determined. Elongation of the coleorhiza was found to be inversely proportional to the amount of air present in the immediate environment, the greatest length being attained in liquid media. Hair formation was pro-

[1]From a dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the Rackham School of Graduate Studies of the University of Michigan, Ann Arbor. Much of this work was done during the tenure of a fellowship granted by the National Science Foundation, Washington, D. C.

portional to the amount of air present and was stimulated by the application of IAA. Lower concentrations of IAA (0.1 to 1.0 mg/lit.) in liquid cultures stimulated coleorhizal elongation, but higher concentrations (5.0 to 10.0 mg/lit.) of this substance proved inhibiting. The greatest length, 10 mm., was obtained in cultures with water and 0.1 mg/lit. IAA. Mitotic figures were not observed in aceto-carmine smears of the coleorhizae of cultured embryos. Coleorhizal growth in these instances, whether characterized by elongation or hair formation, occurred only by the enlargement of existing cells.

TABLE 1

The effect of various factors on the growth of oat coleorhizae.

Treatment	No. of embryos	*Mean coleorhizal length* *Initial*[3]	*Final*	$\frac{\sigma}{\sqrt{n}}$	Length increase	Hairs[4]
20% O_2 + 80% N_2	20	0.8 mm.	2.6 mm.	±0.07	225%	moderate
20% O_2 + 80% N_2 + IAA[1]	21	0.8	2.5	±0.10	212%	considerable
10% O_2 + 90% N_2	20	0.8	3.2	±0.11	300%	slight
4% O_2 + 96% N_2	20	0.8	3.7	±0.07	362%	none
2% O_2 + 98% N_2	20	0.8	5.0	±0.13	525%	none
H_2O	47	0.8	6.1	±0.16	662%	none
H_2O + 0.1 IAA[2]	22	0.8	7.1	±0.29	787%	none
H_2O + 1.0 IAA	27	0.8	7.1	±0.28	787%	none
H_2O + 5.0 IAA	32	0.8	5.7	±0.26	612%	none
H_2O + 10.0 IAA	33	0.8	5.4	±0.22	575%	none

[1]Presoaked 24 hours in water and 10 mg/l. IAA.
[2]The figures given for IAA are milligrams per liter.
[3]Initial lengths based on measurements of section embryos.
[4]Based on the amount of coleorhizal surface covered by hairs.

TABLE 2

The effect of adenine sulfate on the growth of coleorhizae of 0.8 mm. oat embryos.

Medium	No. of embryos	*Mean coleorhizal length* *Initial*	*Final*	Increase in length
White's agar.....	46	0.24 mm.	0.74 mm.*	208%
White's agar + adenine sulfate.....	28	0.24	1.58	558%
White's agar + adenine sulfate + coconut milk..	21	0.24	1.96	771%

*Coleorhizal hairs were produced by some of these embryos.

In order to discover whether elongation would occur in the absence of oxygen, oat seeds also were placed on moist filter paper in several bottles. The oxygen then was removed from the air in the bottles by means of pyrogallol. Coleorhizal elongations were not observed in these cultures and germination was suppressed.

Cultures of detached coleorhizae. Coleorhizae were also dissected from mature embryos and placed in culture on White's agar. In these cultures hairs were formed as in the preceding cultures, but coleorhizal elongation did not occur. Other detached coleorhizae did not grow at all when cultured in White's liquid. It was found that not all coleorhizal cells were capable of forming hairs. The coleorhiza is a hollow organ made up of several layers of parenchymatous cells.

A number of coleorhizae were removed from embryos, slit open and flattened out on nutrient agar. Those which were placed so that the epidermal cells were exposed to the air formed hairs. Others, placed so that the epidermis lay in contact with the agar and the cells of the inner surface were exposed to the air, formed no hairs. Thus it appears that only the epidermal cells are able to produce coleorhizal hairs.

The growth of coleorhizae of smaller embryos. Many small oat embryos were excised from unripened seeds and placed in culture. The largest of these were less than half the size of full term embryos. Embryos in the size range of 1.0 to 1.5 mm. exhibited elongated coleorhizae in liquid media and produced coleorhizal hairs when grown on nutrient agar. Smaller embryos did not develop lengthened coleorhizae in liquid media. Only one instance of coleorhizal elongation was observed in the 125 0.8 mm. embryos grown in White's solution. Yet hairs were usually produced even by 0.8 embryos when they were cultured on nutrient agar. Combinations of certain growth substances were added to White's medium. Yeast extract, IAA and coconut milk had little effect on coleorhizal development. However, in two separate sets of cultures on media containing adenine sulfate, definite coleorhizal elongations and hair suppression were observed. The first of these two sets of cultures was made on White's agar to which 40 mg/lit. of adenine had been added. The second set contained coconut milk in addition to 40 mg/lit. adenine sulfate. Coconut milk was found to promote the overall growth of excised oat embryos but did not of itself stimulate coleorhizal elongation, since marked elongations were not observed in cultures made on coconut milk media without adenine sulfate.

The effect of adenine sulfate on the growth of coleorhizae of 0.8 mm. oat embryos. Not all cultures of 0.8 mm. embryos exhibited coleorhizal elongations when treated with adenine. Larger embryos did not respond to this substance at all. The reason for these failures is not clear at present.

DISCUSSION

The behavior of the oat coleorhiza during germination seems to depend on differences in oxygen tension inside and outside of the seed and its coverings. Low concentrations of oxygen (down to at least 2%) stimulate coleorhizal elongations, while higher concentrations promote the formation of epidermal hairs. Neither elongation nor the formation of the "root hairs" occurred, however, at extremely low concentrations of oxygen. The hairs of the coleorhiza are a regular feature of the surface cells of this structure. That they are not simply adventitious outgrowths is shown by the failure of sub-surface cells to produce them. Indole-3-acetic acid appears not to initiate but rather to determine the extent of such responses as hair formation and coleorhizal elongation.

Skoog and Tsui (1951) report that adenine sulfate may be used to induce differentiation of buds in cultures of tobacco callus on nutrient agar. They also obtained such differentiation without adenine in callus masses submersed in liquid media. Thus adenine sulfate used with surface cultures seems to duplicate the effects obtained with subsurface cultures. Adenine sulfate also seems to substitute for liquid media in embryo cultures at least to the extent that it may induce coleorhizal elongation.

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THE SYNTHESIS OF FIBRINOLYSIN BY FUNGI[1]

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The fungi have long been known to be capable of synthesizing a large number of enzymes, and there are many publications concerning the enzymic activity of fungi but to the best of my knowledge little or no work has been published on the fibrinolytic activity of fungi.

The fibrinolytic activity of hemolytic streptococci has been extensively studied by Tillet and Garner (1933), who reported that all 28 strains of hemolytic streptococci associated with human infections formed fibrinolytic substances which liquefied human blood clot while only 3 out of 18 hemolytic streptococci of animal origin were capable of causing fibrinolysis.

Van Deventer and Reich (1934) observed that fibrinolysin produced by hemolytic streptococci dissolves human but not animal plasma clots with the exception of rhesus plasma.

Madison (1934) studied fibrinolytic streptococci from lower animals and showed that an organism isolated from a particular animal species had a greater activity on the fibrin of that animal species than on the fibrin of another species.

Neter and Witebsky (1936), who studied fibrinolytic activity of hemolytic streptococci and other microorganisms observed that fibrinolysin production is not limited to hemolytic streptocci alone, if the sugar content of the culture medium is increased. They showed that other microorganisms such as *Streptococcus viridans*, *Enterococcus*, *B. coli*, *B. lactis*, etc. are capable of producing fibrinolysin when cultivated in 2 percent glucose broth but not in 0.5 percent glucose broth.

This present study is an attempt to find out whether fungi have the ability of synthesizing fibrinolysin. This work is a preliminary survey of species and strains of fungi in connection with their ability to synthesize fibrinolysin. The effect of certain environmental conditions on the production of the enzyme was also studied.

MATERIALS AND METHODS

The preliminary testing for ability to synthesize fibrinolysin was made by the technique described by Crabill and Reed (1915). The following medium has been used throughout the investigation.

$MgSO_4$	0.5 gm.
KCl	0.5 gm.
K^2HPO_4	1.0 gm.
Trace mineral salts	1 ml.
Ox blood fibrin	10 gm.
Agar	20 gm.
Distilled water	1000 ml.

Trace mineral salts dil. 1: 1000

$FeSO_4$	.04 g,./lit.
$ZnSO_4$	.025 gm./lit.
$MnSO_4$	.025 gm./lit.
$Na_2B_4O_7$	.025 gm./lit.
NH_4MoO_3	.025 gm./lit.
$CuSO_4$	.025 gm./lit.

The above culture medium was prepared by dissolving the salts in a portion of distilled water and then making to volume; blood fibrin was then added by

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mixing in a Waring Blender in order to obtain uniform distribution through the medium. Agar was added and the medium was sterilized at 15 lbs. pressure for 20 min. Plates were poured directly from the flask with frequent shaking between pourings to prevent the settling of the finely-divided blood fibrin. Upon cooling, the medium was opaque due to the presence of the many fine particles of fibrin. Plates were inoculated by introducing spores at a single, central point on the plate. If a fungus is capable of synthesizing fibrinolysin, a clear area appears under the developing colony and sometimes with slow-growing fungi, a clear halo appears around the colony.

TABLE 1

Organisms used

STOCK NO.	
I–28	*Acrostalagmus cinnabarium* Corda
8	*Ascobolus striisporus* (Ellis & Dearn) Seaver
25	*Ascobolus striisporus* (Ellis & Dearn) Seaver
27	*Ascobolus striisporus* (Ellis & Dearn) Seaver
33	*Ascobolus striisporus* (Ellis & Dearn) Seaver
2	*Aspergillus fischeri* Wehmer
4	*Aspergillus fischeri* Wehmer
7	*Aspergillus (flavus oryzae)*
9	*Aspergillus fumigatus* Fresenius
10	*Aspergillus niger* Van Tieghem
14	*Aspergillus oryzae* (Ahlburg) Cohn.
15	*Aspergillus tamarii* Kita.
16	*Aspergillus terreus* Thom
17	*Aspergillus terreus* Thom
18	*Aspergillus terreus* Thom
19	*Aspergillus ustus* (Bainier) Thom, Church.
I–48	*Beauveria bassiana* (Bals.) Vuill.
I–41	*Bispora punctans* (Schw.) Hughes
23	*Coprinus sp.*
21	*Chaetomium globosum* Kze.
205	*Circinella* sp.
I–58	*Colletotrichum phomoides* (Sacc) Chester
I–42	*Coniothyrium fuckeli* Sacc.
I–45	*Cordana americana* Hughes
205	*Cunninghamella* sp.
I–4	*Curvulvaria* sp.
I–53	*Dendryphiopsis* atra
I– 5	*Fusarium lycopersici* Sacc.
I–31	*Gliocladium deliquescens* Sopp.
I–30	*Gliocladium roseum* Bain
116	*Hansenula ciferri* Lodder
I– 8	*Helminthosporium sativum* Pammel
I– 9	*Heterocephalum aurantiacum* Thaxt.
I–10	*Memnoniella echinata* (Rivolta) L.
I–37	*Melanconium fuligineum* Cav.
24	*Metarrhizium glutinosum* Pope
5	*Mucor* sp.
I–12	*Oidium lactis* Fres.
I–13	*Pestalozzia* sp.
I–14	*Phoma destructiva* Plowr.
69	*Phycomyces blakesleeanus* Burgeff
I–16	*Sclerotinia fructicola* (Wint) Rehm.
I–63	*Sclerotium rolfsii* Sacc.
37	*Sordaria humana* (Fekl)
I–19	*Spegazzinia* sp.
38	*Stachybotrys atra* Corda
I–21	*Stemphylium* sp.
I–23	*Trichoderma viride* A. et S.
I–25	*Verticillium puniceum* (Cook et Ellis) Grev.
206	*Zygorhynchus* sp.

For the initial screening experiments cultures were incubated at room temperature. Measurements of colony and clear zone diameters were made at 48 hr. and 72 hr. and each experiment was terminated at the end of this period. The average measurement was computed from two measurements at right angles to each other.

The organisms used were obtained from Dr. W. D. Gray, Department of Botany and Plant Pathology, The Ohio State University, and are listed in table 1 with their stock culture numbers.

The effect of sugar on the production of fibrinolysin was studied by using the above medium containing 2 percent dextrose.

The relation of temperature to fibrinolysin production was also determined. For this purpose the plates were inoculated and incubated at 15°C, 20°C, 21–25°C, 30°C, and 35°C.

The influence of pH of the medium on the production of fibrinolysin was also investigated. The initial pH of the medium was adjusted with N/1 HCl or N/1 NaOH before autoclaving and the final measurement was made with a Beckman Model H pH meter after autoclaving. Although all precautions were taken in making the medium, slight differences in pH were evident in each series. The six media termed a series were as follows: pH 3.2, 4.2 5.5, 6.2, 7.2, and 7.8. Each series was incubated at 25° and 30°C. All measurements reported represent the average of at least 9 replicated plates.

EXPERIMENTAL RESULTS

Screening experiments. The results of these experiments show that 23 out of the 50 fungi studied are capable of synthesizing considerable amounts of fibrinolysin. The organisms may be placed into three groups on the basis of the relative amounts of growth and fibrinolytic activity. The first group is characterized by rapid mycelial growth which extends well beyond the zone of fibrinolytic activity. The fungi falling into this group are (8, 25, 33, 23, 21, and 37) *Ascobolus striisporus*, *Ascobolus striisporus*, *Ascobolus striisporus*,* *Coprinus* sp., *Chaetomium globosum*, *Sordaria humana*. In the second group the clear zone free of blood fibrin extends beyond the edge of the colony, but the rate of growth is slow: (7, 14, 15, 16, 17, I–58, I–42, I–30, I–10, and 24) *Aspergillus* (*flavus oryzae*), *Aspergillus oryzae*, *Aspergillus tamarii*, *Aspergillus terreus*, *Aspergillus terreus*, *Colletotrichum phomoides*, *Coniothyrium fuckeli*, *Gliocladium roseum*, *Memnoniella echinate*, *Metarrhizium glutinosum*, are in this group. The third group is intermediate between the above two, the colony and clear zone being equal in size. The organisms of this group are (I–5, I–31, I–19, 38, I–21, and I–23) *Fusarium lycopersici*, *Gliocladium deliquescens*, *Spegazzinia* sp., *Stachybotrys atra*, *Stemphylium* sp., *Trichoderma viride*. The results are summarized in table 2.

The first and third groups do not produce a halo on blood fibrin-agar but grew well and dissolved blood fibrin in the agar which was in contact with the mycelium. These are considered by Crabill and Reed as weak enzyme producers, and the presence of a halo around the edge of a fungus colony is indicative of stronger ensymic activity.

The effect of sugar content of the medium on the production of fibrinolysin. For studies on the effect of sugar on fibrinolysin production, 18 different fungi were used, and it was found that dextrose inhibits the formation of fibrinolysin by most of the fungi studied. The degree of inhibition varies with the organism. The results of this experiment as shown in table 3 indicate that Cultures 21, I–42, I–5, I–31, I–10, I–19, 38, I–21, and I–23 produce no fibrinolysin in the medium containing 2 percent dextrose, and Cultures 8, 25, 27, 33, 15, 16, 23, and 37 exhibit only traces of fibrinolysin. Culture I–30 produces more fibrinolysin in the presence

*Three different monospore strains were used.

of dextrose than in dextrose-free medium. The sugar content of the medium apparently has no effect on the production of fibrinolysin by Culture 24. In all cases growth is greater when cultivated in 2 percent dextrose medium.

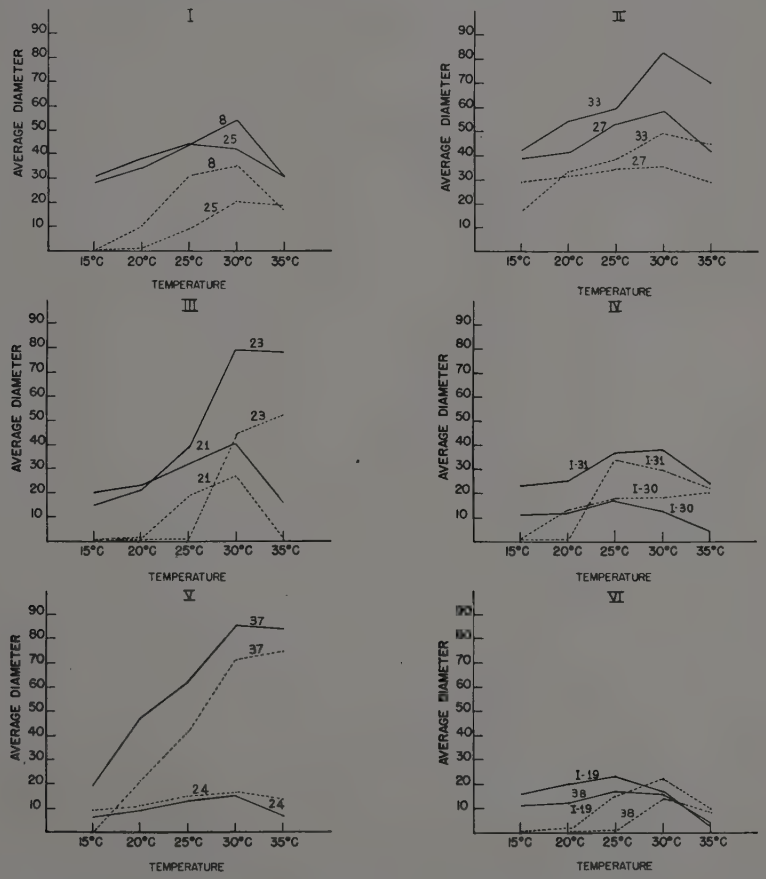
TABLE 2

Average diameter in millimeters of colony and zone of fibrinolytic activity of all fungi studied

STOCK NO. OF ORGANISM	48 HRS.		72 HRS.	
	COLONY	CLEAR ZONE	COLONY	CLEAR ZONE
I-28	–	–	+	–
8	35.31	26.61	48.84	41.96
25	24.96	14.5	31.82	18.9
27	48.46	36.59	65.27	51.86
33	45.03	31.75	64.52	49.0
2	17.67	+	28.57	+
4	12.28	–	19.97	–
7	14.63	21.69	35.2	42.55
9	18.35	+	27.87	+
10	10.0	–	13.45	–
14	12.28	17.43	20.78	26.87
15	13.5	16.06	21.08	25.44
16	11.56	16.06	15.42	19.09
17	13.25	+	18.71	+
18	11.53	15.09	15.8	22.06
19	15.59	–	22.62	–
I-41	–	–	+	–
I-48	–	–	+	–
23	37.06	20.03	61.25	34.37
21	32.21	17.18	49.7	38.25
205	32.79	–	37.0	–
I-58	6.58	11.5	16.08	19.41
I-42	8.75	13.08	13.08	19.16
I-45	10.0	–	16.66	–
204	60.7	–	70.0	–
I- 4	19.33	–	29.83	–
I-53	–	–	+	–
I -5	23.16	23.16	37.33	37.33
I-31	29.65	29.65	43.28	43.28
I-30	11.93	14.53	18.65	21.46
116	–	–	+	–
I- 8	15.83	–	29.0	–
I- 9	9.3	+	14.27	+
I-10	8.37	11.25	13.25	17.75
I-37	9.0	–	16.66	–
24	9.87	14.75	14.82	20.88
5	34.55	+	54.85	+
I-12	17.33	–	24.0	–
I-13	22.25	–	34.33	–
I-14	22.85	–	33.25	–
69	12.1	+	17.0	+
I-16	–	–	–	–
I-63	38.33	–	57.66	–
37	64.0	48.07	90.0	90.0
I-19	14.23	14.23	20.55	20.55
38	10.45	10.45	15.35	15.35
I-21	20.0	20.0	27.83	27.83
I-23	37.15	37.15	47.25	47.25
I-25	3.91	+	8.75	+
206	43.4	–	69.25	–

+ Denotes slight growth and slight fibrinolytic activity.
– Denotes no growth and no fibrinolytic activity.

The influence of temperature. Twelve fungi were used in the temperature studies and all grew throughout the temperature range used. The optimum for growth is 25°–30°C. Essentially no fibrinolysin was formed by most of the fungi at 15°C with the exception of Cultures 27, 33, 24, and 37 which are capable of synthesizing fibrinolysin at this temperature. In most cases, the optimum temperature for the production of fibrinolysin is at 30°C.



FIGURES 1 to 6. Relation of temperature to growth and fibrinolysin production by fungi. All measurements made after 72 hr. incubation except those for culture 37 (48 hr.) Unbroken line indicates colony diameter, broken line represents diameter of clear area.

The behavior of I–19 and I–30 differs from the others in that the optimum temperature for growth is at 25°C ,while the optimum for formation of fibrinolysin lies at 35° and 30°C. The production of fibrinolysin increases with increase of temperature from 15° to 35°C, while growth decreases with increase of temperature from 25° to 35°C. The results are summarized in table 4 and expressed graphically in fig. 1 to 6.

The effect of pH. The data represented in table 5 indicates that the range of pH that will permit growth and formation of fibrinolysin depends on the individual organism, the initial reaction of the medium and the temperature. In the majority of cases growth and production of fibrinolysin occur at temperatures of 25° and 30°C within the range of pH 4.2 to 7.8. At a temperature of 30°C there is a complete inhibition of growth and formation of fibrinolysin in all cases at pH 3.2 with the exception of Culture 37 which exhibited growth and fibrinolytic activity over a wide pH range (3.2 to 7.8).

Cultures 24 and 33 showed growth and fibrinolytic activity within the range of pH 3.2 to 7.8 at a temperature of 25°C and pH 6.2 is the optimum at both temperatures incubated.

TABLE 3

The effect of sugar content of the medium on growth and production of fibrinolysin by fungi. Average diameter in millimeters of the colony and clear zone in 72 hours.

STOCK NO OF ORGANISMS	SUGAR-FREE MEDIUM		MEDIUM CONTAINING 2% DEXTROSE	
	COLONY	CLEAR ZONE	COLONY	CLEAR ZONE
8	51.73	40.30	90.0	+
25	48.75	26.0	63.5	+
27	77.58	54.54	90.0	+
33	63.05	47.0	85.0	+
15	15.0	20.0	31.0	+
16	16.5	18.25	18.25	+
23	59.25	24.33	72.25	+
21	46.29	32.12	46.66	−
I-42	13.08	19.16	18.58	−
I- 5	37.33	37.33	42.83	−
I-31	43.62	43.62	90.0	−
I-30	19.41	21.04	17.07	22.5
I-10	13.25	17.75	13.75	−
24	14.8	19.07	18.03	19.45
37	90.0	90.0	90.0	+
I-19	20.45	20.45	26.3	−
38	15.16	15.16	14.65	−
I-21	27.5	27.5	31.91	−
I-23	48.20	48.20	90.0	−

+ Denotes slight growth and slight fibrinolytic activity.
− Denotes no growth and no fibrinolytic activity.

The optimum pH for growth and production of fibrinolysin by Cultures 8 and 27 is shifted to a less acid reaction at higher temperatures.

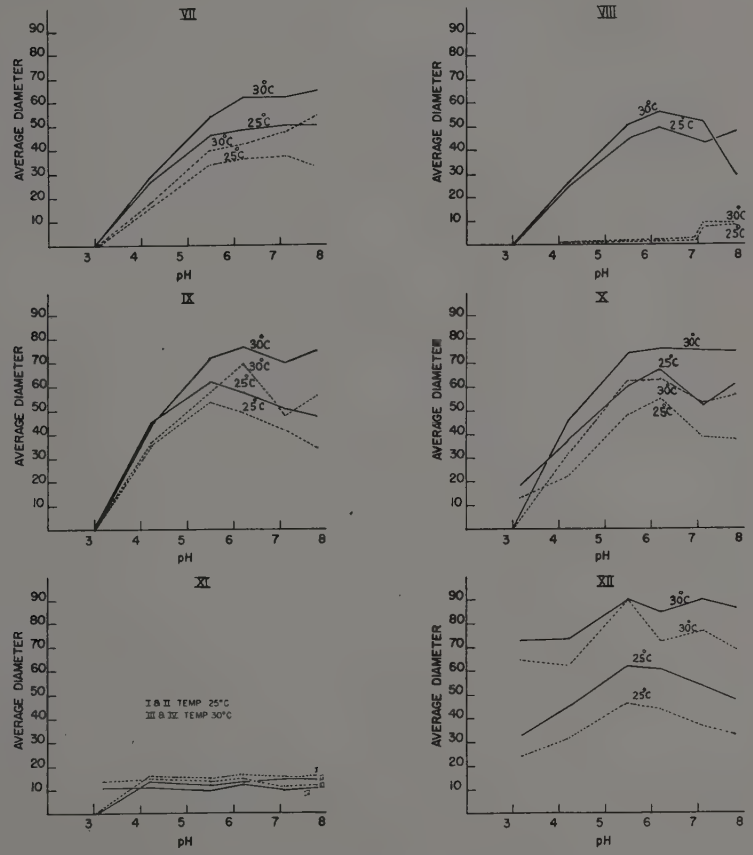
In all cases growth and fibrinolytic activity are greater at 30°C than at 25°C within the same range of pH.

The data are represented graphically in figures 7 to 12.

DISCUSSION

From the preceding results it is obvious that some fungi are capable of synthesizing an extracellular enzyme which digests blood fibrin; this enzyme is known as fibrinolysin. The extent of fibrinolytic activity depends upon the type of organism. In general, the slow-growing ones appear to have strong fibrinolytic activity while those which are rapid growers have weak fibrinolytic activity. The accumulation of fibrinolysin by the fast growing ones appears to be greater than the slow-growing organisms in a definite period of time.

Different species and strains of fungi behave differently on medium containing sugar. Waksman (1918) reported that the sugar content of the medium has no decided influence upon the production of proteolytic enzyme by *Aspergillus niger*.



FIGURES 7 to 12. The relation of pH to growth and fibrinolysin activity of fungi at 25° and 30° C. All measurements made after 72 hr. except those for culture 37 (48 hr.) Unbroken line indicates colony diameter, broken line represents diameter of clear area.

The work of Jones (1916) showed that *B. proteus* will not form gelatinase on gelatin when glucose is present, but as soon as the glucose has been used up the gelatinase appears. He concluded that this enzyme is elaborated in response to need.

Neter and Witebsky (1936) pointed out that *Streptococcus virdians* and Enterococcus did not show any fibrinolytic activity when cultured in 0.05 percent glucose-broth but produced the fibrinolytic substance in 2 percent glucose-broth.

From the result of this work it appears that the effect of dextrose on the production of fibrinolysin varies with species and strains of fungi studied.

TABLE 4

Average diameters in millimeters of colonies and clear zones at different temperatures

Stock No. of Organism	Incubation Period	15° C		20° C		25° C		30° C		35° C	
		Colony	Clear-zone	Colony	Clear-zone	Colony	Clear-zone	Colony	Clear-zone	Colony	Clear-zone
8	48 hrs.	18.38	+	20.88	8.33	28 38	15 0	36 25	22.33	22.88	11.22
	72 "	31.33	+	38.91	10.0	44 66	31.58	54.91	35.12	31.75	17 16
25	48 "	19 42	−	21.05	+	28 0	7 83	27 83	8.61	19.77	9.77
	72 "	28.33	−	34.95	+	44.27	9.58	42.89	20 55	31.75	19.91
27	48 "	31.11	18.0	34.42	19.72	35.05	20 77	37.94	22.33	28.56	20.12
	72 "	39.0	29.16	41.25	31.16	53.96	34.5	58 16	35.51	41.70	29.58
33	48 "	28 38	11.75	37.66	19.33	44.0	28.5	55.5	43.0	37.94	25.16
	72 "	42.0	17.5	54.5	33.91	59.62	38.75	82.08	49.0	70.58	44.16
21	48 "	13.66	+	14 5	+	17.61	10 66	22.5	11 71	12.41	+
	72 "	20 97	+	23 86	+	32.0	19.77	40 61	27.16	16.93	+
23	48 "	8.22	−	9.88	−	25.05	+	49.83	19.61	55.55	28.88
	72 "	15.0	−	21.0	−	39.38	+	79.94	44.0	78.38	52.66
I-31	48 "	15 61	+	18.29	+	25.88	20 08	30.2	27.0	17.58	15.61
	72 "	23 38	+	25.17	+	37.94	34.25	38.88	30.16	24.31	22.5
I-30	48 "	7 88	+	8.81	9.20	11.61	13 26	10 30	15.0	3.05	16 33
	72 "	11 66	+	12 83	13 33	17.25	18.46	13.88	18.5	4.22	20.08
24	48 "	4.8	7.61	6.27	9.08	8.05	10.61	9.13	11.33	6 02	11.27
	72 "	6.62	9.08	9.83	11.16	13.08	15.41	15.73	17.58	7.54	14.75
37	48 "	19.41	+	47.1	26.6	62.68	41.25	85 66	71.88	83.12	74.0
	72 "	40.5	16.54	68 66	52.75	90.0	90 0	90.0	90.0	90.0	90 0
I-19	48 "	11.16	−	15.61	+	18.0	9.72	9.69	10.83	3.44	8.77
	72 "	16.61	−	20.97	+	23.22	15.5	17.72	22.66	3.5	10.22
38	48 "	5.41	−	6.66	−	10.25	+	9.08	8.88	3.91	5.87
	72 "	11.16	−	12.22	−	17.05	+	16.27	14.05	4.09	8.12

+ Denotes slight growth and slight fibrinolytic activity.
− Denotes no growth and no fibrinolytic activity.

TABLE 5

The relation of pH in relation to growth and production of fibrinolysin by 6 fungi. Based on the average diameter in millimeters of colony and clear zone in 72 hr.

Stock No. of Organisms	Temp.	pH 3.2		pH 4.2		pH 5.5		pH 6.2		pH 7.1		pH 7.8	
		Colony	Clear-zone	Colony	Clear-zone	Colony	Clear-zone	Colony	Clear-zone	Colony	Clear-zone	Colony	Clear-zone
8	25° C.	–	–	26.79	17.54	47.42	34.28	49.33	37.0	51.04	38.33	49.88	34.99
	30° C.	–	–	30.92	17.78	55.5	40.33	63.11	43.5	63.29	48.7	66.27	55.0
25	25° C.	–	–	25.94	+	44.33	+	49.88	+	43.64	8.0	48.16	8.33
	30° C.	–	–	26.41	+	50.92	+	56.93	+	52.56	9.9	29.73	9.07
27	25° C.	+	–	45.16	36.55	62.61	53.13	58.64	49.88	51.11	42.16	48.11	37.33
	30° C.	–	–	44.91	37.5	72.38	59.44	77.5	69.83	70.05	48.05	75.5	57.27
33	25° C.	19.5	13.87	38.5	22.44	60.55	48.22	67.16	55.41	52.77	39.66	61.27	38.81
	30° C.	–	–	46.81	32.37	74.40	62.5	76.58	63.75	75.11	53.47	75.35	57.0
24	25° C.	11.72	14.16	11.66	15.11	10.8	14.94	12.44	15.22	10.05	12.88	11.38	12.5
	30° C.	–	–	10.15	14.5	12.33	15.94	14.77	17.44	15.05	16.94	15.61	16.22
37	25° C.	75.83	61.5	79.5	71.5	82.87	82.0	81.37	72.62	77.87	67.28	73.5	55.28
	30° C.	81.3	75.5	90.0	90.0	90.0	90.0	90.0	90.0	90.0	90.0	90.0	90.0

+ Denotes slight growth and slight fibrinolytic activity.
– Denotes no growth and no fibrinolytic activity.

Dextrose inhibits the formation of fibrinolysin by most of the fungi, but some produce more fibrinolysin in the presence of dextrose, and others do not appear to be affected by the presence of dextrose. The results of this work do not agree with those of Waksman which concerned only *Aspergillus niger*.

It is quite probable that the presence of a substance which is more easily decomposed than blood fibrin hinders fibrinolysin formation. The degree of inhibition depends upon the type of organism. The presence of substances which diminish the utilization of a particular nutrient in the substrate brings about a diminution in the production of the corresponding enzyme.

Temperature influences the enzymic processes in general parallel with the rate of growth. It is evident from this experiment that growth and formation of fibrinolysin increases with increase in temperature up to 30°C and declines at 35°C while the optimum lies at 25° to 30°C. The decrease of fibrinolytic activity with the decrease of temperature may be due to the fact that the time required for the accumulation of fibrinolysin in sufficient quantity to start the liquefaction of blood fibrin is longer at low temperature than at high temperature. Inhibition of the production of fibrinolysin occurs at 15°C by most of the fungi studied with the exception of Nos. 27, 33, 24, and 37 (*Ascobolus striisporus*, *Ascobolus striisporus*, *Metarrhizium glutinosum*, and *Sordaria humana*) which exhibit fibrinolytic activity at a temperature as low as 15°C.

Waksman (1918) studied the proteolytic enzymes of soil fungi and actinomycetes, and observed that a temperature of 29°C to 34°C is optimum for enzyme activity of *Aspergillus niger* and that the activity dropped at 39°C. This observation by Waksman coincides with that made in the present studies.

The pH limit for growth and production of fibrinolysin is influenced by temperature as shown in this investigation since pH 3.2 inhibits growth and formation of fibrinolysin at 30°C. The pH range is widest at 25°C. In some cases, at the higher temperature the optimum pH for growth and fibrinolytic activity is higher than at lower temperatures. All fungi showed greater growth and fibrinolytic activity at 30°C than at 25°C within the same range of pH.

Wolpert (1924) working with the growth of wood-destroying fungi in relation to hydrogen-ion concentration stated that the range of pH in which those fungi will grow and the amount of mycelial growth depend upon the individual organism, the composition of nutrient solution, the initial active acidity and the temperature. The major portion of the growth curves of all of these fungi is on the acid side of neutrality and in the majority of cases wholly on the acid side.

Karrer (1921) working with the effect of hydrogen-ion concentration upon the accumulation and activation of amylase produced by certain fungi concluded that a relation, which varies with the organism seemed to exist between the hydrogen-ion concentration of the medium and the accumulation of extra- and intra-cellular amylase. An optimum zone of activity between pH 3.0 and pH 6.0 existed for *Penicillium italicum*, while in the other fungi the optimum was more sharply defined at 6.0. Inactivation occurred on the alkaline side.

Brancato and Golding (1933) in their studies of the influence of pH on the diameter attained by colonies of *Aspergillus niger*, *A. flavus*, *Penicillium notatum*, *P. expansum*, *P. roqueforti*, and *Geotrichum candidum* reported that pH 5.5 is optimum or very near optimum for all of these organisms.

Webb (1921) in his studies of the germination of spores of certain fungi in relation to hydrogen-ion concentration observed that inhibition of growth in all cases occurred at pH 3.0.

The relation of temperature to the effect of hydrogen-ion concentration (Wolpert, 1924) showed that the pH range of *Armillaria mellea* is 2.5 to 7.5 at 15°C, 2.0 to 7.8 at 25°C and 2.5 to 7.4 at 35°C when cultivated on a sucrose-peptone medium.

The finding of the present studies is similar to that of Wolpert in that the

pH range in which the fungi will grow depends on the individual fungus, the initial reaction of the medium and the temperature.

SUMMARY

1. Fifty fungi have been surveyed for their ability to synthesize fibrinolysin, using an *invivo* method described by Crabill and Reed. Of all fungi investigated only 23 species and strains are capable of synthesizing a considerable amount of fibrinolysin.

2. The sugar content of the medium show a marked inhibitory effect on the production of fibrinolysin by most of the fungi.

3. The optimum temperature for the production of fibrinolysin lies between 25° and 30°C and inhibition occurs at 15°C in most cases. Growth takes place over a wide range of temperature (15° to 35°C).

4. Temperature influences the pH limits for growth and formation of fibrinolysin. At 30°C inhibition occurs at pH 3.2. Under favorable temperatures (about 25°C) pH range is widest for growth and fibrinolytic activity. The optimum pH for the majority of fungi is between pH 5.5 and 7.1.

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I wish to express my deep gratitude to Dr. W. D. Gray, under whose direction this work was conducted, for his encouragement, constructive criticism, and valuable suggestions.

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STABILITY OF VITAMIN C IN TOMATO JUICE

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The scientific literature contains many references pertaining to factors influencing the nutritive value of fruits and vegetables. A considerable proportion relate to the effect of processing and of storage on the vitamin content of these foods.

The nutritive value of tomato juice depends largely on its ascorbic acid content. Prevention of the loss of ascorbic acid through enzymatic oxidation is one of the most difficult problems. Naito and Isimaru (1938) reported the presence of ascorbase in tomatoes. Later, Wokes *et al.* (1943) showed that the oxidase activity is greatest in both the unripe and ripe skin and the least in the juice. Kardo-Sysoeva *et al.* (1938) pointed out the presence of a stabilizer or a protective factor for ascorbic acid in the tomato pulp. The same investigators also showed that such stabilizer may be inactivated by boiling. On the other hand, Griffee (1943) observed no losses of vitamin C during preparation of tomato juice.

The present work is a part of a comprehensive program of study concerning the behavior, adaptability, composition, crop yield, etc., of many imported varieties of tomatoes. This work has been conducted to find out the rate of vitamin C destruction in tomato juice pressed out of fully ripe preheated and non-heated tomatoes. The heating was carried in live steam for either five or fifteen minutes.

MATERIALS AND METHODS

Samples of 6 imported varieties of tomatoes, raised on the Faculty of Agriculture farm, namely New Stone, Sunrise, Norton, Victor, Tomato California First and Early, and Sutton's Best of All, were used in this work. Samples were picked, sorted and stored at 5°C till the next day and, then, analysed for ascorbic acid content over a period of two hours. The method used was that described by the Association of Vitamin Chemists, Inc. (1951). Ascorbic acid content was expressed as mg. per 100 g. tomato juice. In this investigation, samples of 16-20 tomato fruits were used for analysis as recommended by Mabrouk (1950).

EXPERIMENTAL RESULTS

Tables 1 and 2 and figures 1 and 2 show the average results of three experiments.

DISCUSSION

The results show a gradual but slow decrease in the vitamin C content of the non-heated juice; in the 6 tested varieties the decrease ranged from 30 to 65 percent over a period of 2 hr. On the other hand, heating caused a sudden large decrease in the juice amounting to approximately 90 percent. The residual reducing effect of the pre-heated juice remained constant over the tested period.

The gradual and rather slow rate of Vitamin C destruction in the non-heated tomato juice probably indicate the presence of a vitamin C stabilizer. In the meantime, the sudden decrease in the vitamin C content of juice pressed out from pre-heated tomatoes might indicate the destruction of such a stabilizer. The residual reducing value of the juice after heating may be due to the presence of the so-called reductones as suggested by Roe *et al.* (6).

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TABLE 1

Stability of Vitamin C in Tomato Juice at Room Temperature

Time Minute	Ascorbic acid mgm/100 gm. New Stone	Sunrise	Norton	Victor	Tomato Calif. First and Early	Sutton's Best of All
0	18.02	19.43	20.91	14.43	24.85	26.75
5	16.90	18.44	19.85	10.77	22.67	25.27
10	16.90	18.22	19.71	9.29	21.89	24.78
15	15.98	17.95	19.50	8.25	21.33	24.50
20	15.91	17.74	19.36	8.17	20.63	24.50
30	15.49	17.60	19.08	7.88	20.42	24.08
40	14.92	17.25	18.58	7.46	20.06	24.08
50	14.92	16.90	17.74	6.83	19.85	23.23
60	14.36	16.60	17.32	6.62	19.01	23.23
75	14.08	16.32	16.61	6.48	18.75	22.81
90	13.60	16.04	16.05	5.42	18.09	22.67
105	13.09	15.12	15.98	5.00	17.32	22.13
120	12.67	14.08	15.28	.....	16.33	.....

TABLE 2

Stability of Vitamin C in Heated Tomato Juice

Time Minute	Ascorbic acid mgm/100 gm. Norton (a) 20.91 mgm/100 gm before heating	Victor (b) 14.43 mgm/100 gm before heating
0	4.05	1.40
5	4.05	1.40
10	4.05	1.40
15	4.05	1.40
20	4.05	1.40
30	4.05	1.40
40	4.05	1.40
50	4.05	1.40
60	4.05	1.40
75	4.05	1.40
90	4.05	1.40
105	4.05	1.40
120	4.05	....

(a) The fruits were heated for five minutes before juice extraction.
(b) The fruits were heated for fifteen minutes before juice extraction.

SUMMARY

A slow rate of vitamin C destruction was observed in the case of non-heated tomato juice; while pre-heating the fruits was accompanied by a sudden great decrease in their ascorbic acid content (approximately 90%).

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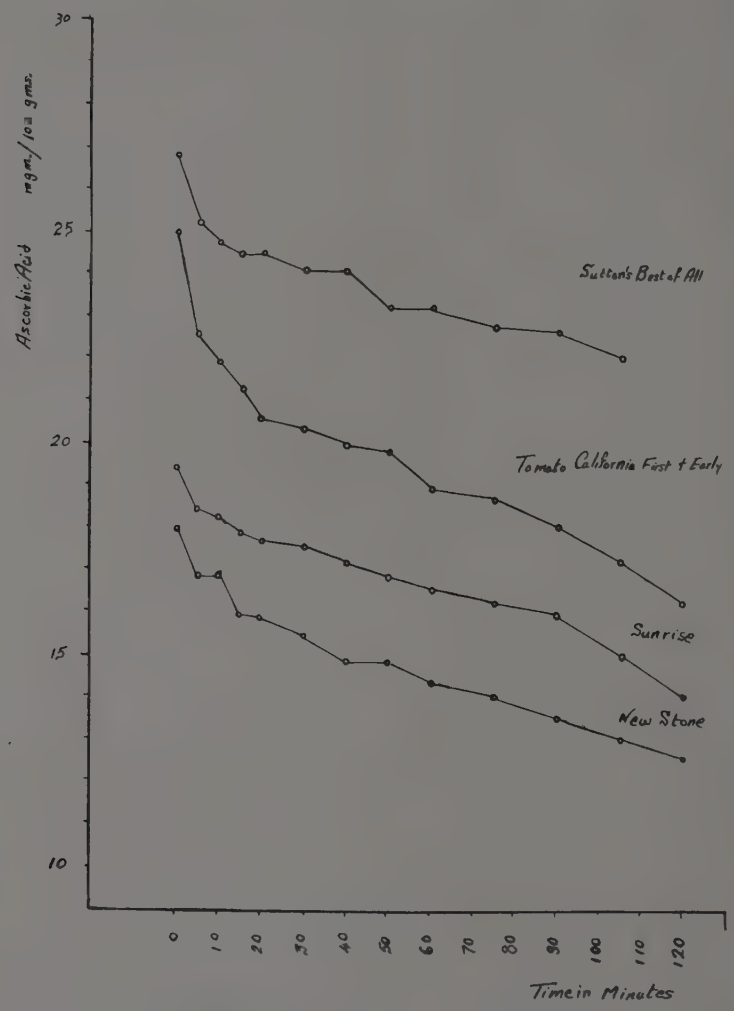

FIGURE 1. Ascorbic Acid Destruction in Non-heated Tomato Juice.

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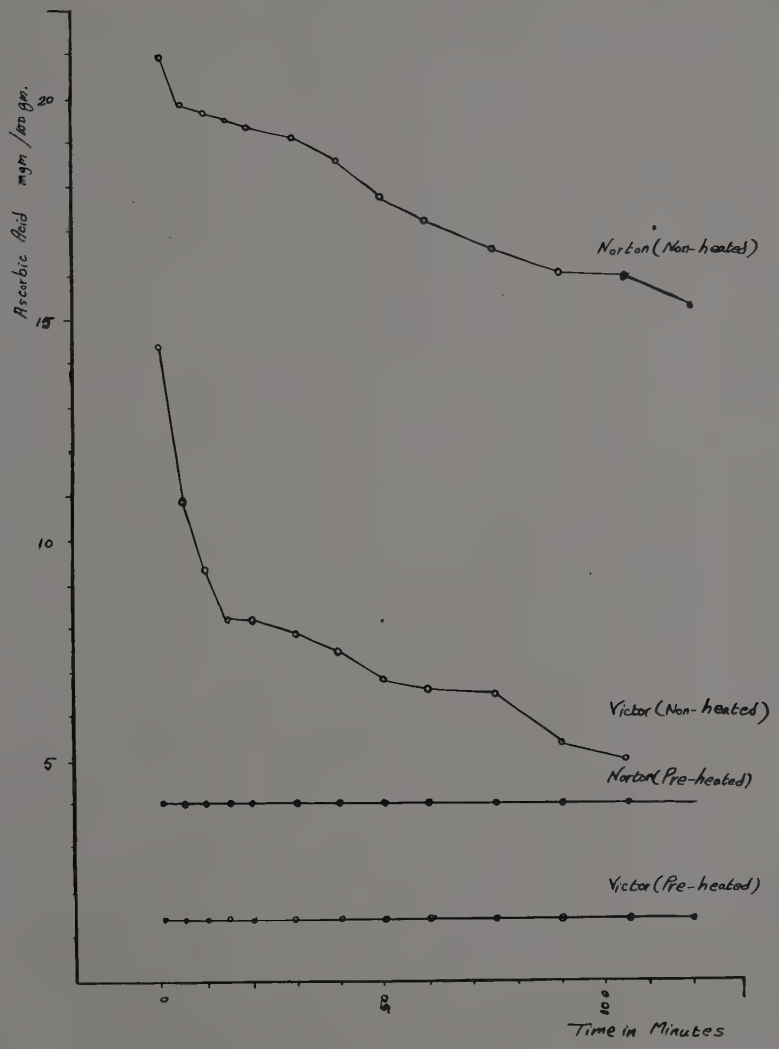

FIGURE 2. Ascorbic Acid Destruction In Non-heated and Pre-heated Tomato Juice.

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ANIMAL REMAINS FROM OHIO ROCK SHELTERS

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A number of rock shelters in Ohio have been examined for archaeological remains over a period of many years, and some of these have been reported in publications. With one or two exceptions little attempt has been made to identify more than the usual, commonly found and easily identified species like the deer, elk, bear, raccoon, and wild turkey. As there has accumulated from excavations representative collections from several rock shelters, we were interested to know just what kinds of mammals and birds were recovered and are available in our Ohio State Museum collection.

To bring this material up to date as well as examine all of the material available, the rock shelter animal remains were recently studied and additional identification was made where possible. The remains from Ash Cave excavated by the writer in 1928 had already been studied, as well as those from Twinsburg rock shelter in Summit county. What remained to be studied was the material from Kettle Hill shelter and the mammal remains from Canter Caves; also the re-examination of material excavated by Dr. Wm. C. Mills at Boone and Buzzard rock shelters in Jackson county in 1905.

The animal bone material in the rock shelters is not numerous as compared with that found in the village sites. When one considers the limited space in these shelters and the fact that only a small number of persons camped or lived in them at a time, one would not expect the accumulation to be very great. There is also the possibility that much of the bone material was merely tossed out of the shelters and washed down the slopes below.

A knowledge of what kinds of animals are represented from the bone material left in the shelters by the Indians is of interest for several reasons. Several kinds of animals, particularly birds, were living in Ohio in the early days that no longer are here and some are extinct. It gives some idea of the former fauna of a region and may be the only means of securing definite examples of occurrence; the last source of collecting *actual specimens* or *parts* of extinct species, that once occurred in Ohio. To the archaeologist it gives some information on the food secured by the Indians, as well as the kinds of animals killed for their fur, feathers or bones used for tools, implements, and ornaments.

The shelters included in this study are Ash Cave in Hocking county, Kettle Hill in Fairfield county, Boone, Buzzard and Canter Caves in Jackson and Twinsburg rock shelter in Summit county. All of these shelters have some animal bone remains in varying amounts and only three of these have been completely excavated. These are the two Canter Caves, namely "Indian" and "Echo" caves, and the Twinsburg rock shelter.

ASH CAVE

Ash Cave is perhaps the most widely known of all the rock shelters in Ohio and is located about four miles east of Bloomingville, Benton Township, Hocking county. It is situated on the west side of a steep ravine, at the head of which is a much larger "wet cave" and waterfalls. The ravine is a scenic area and is now a State Park which is visited throughout the year, the public attractions being the scenic beauty of the area and the large "wet cave" at the head of the hollow.

The first examination of Ash Cave was made about 1876 by E. B. Andrews for the Peabody Museum and published in 1877 in the Tenth Annual Report of the Peabody Museum. In his report Andrews (1877) mentions animal remains as follows:

"It was a confused mass of sticks for arrows, stalks of coarse grasses, *food bones in great variety*, bits of pottery, flints, nuts, corn cobs etc."

Later examination was made by Warren K. Moorehead and Thomas Wilson (1895). Among other things mentioned as found by them are "*bones hardened by fire.*"

In April 1928 the writer spent three days in making a test trench in the floor of the cave and in an area 8 x 10 feet all of the animal bones now available for study were found. These represent bones of the following animals:

Mammals

Black Bear	2
Skunk	2
Wild Cat	1
Gray Squirrel	3
Rabbit	1
Elk	1
Deer	124

Birds

Duck	1
Wild Turkey	8
Passenger Pigeon	1

Reptiles

Box Turtle	13

Of interest in this small series of bones is the greater number of deer bones, and the finding of the Passenger Pigeon.

KETTLE HILL CAVE

This cave is perhaps one of the largest in the area which has been known for many years to contain Indian artifacts and has been excavated by local collectors. In the spring of 1927 the writer and the late H. C. Shetrone examined a small portion of the cave. Later Dr. E. F. Greenman and the writer examined another part of the cave and all material recovered from these excavations as well as that found earlier by the writer are now in the Ohio State Museum collection.

Among the animal remains from this shelter the following have been identified:

Mammals

Opossum	7
Short-tailed Shrew	1
Black Bear	3
Raccoon	7
Mink	2
Skunk	2
Dog	17
Gray Fox	1
Wild Cat	3
Woodchuck	7
Chipmunk	1
Gray Squirrel	23
Fox Squirrel	1
Flying Squirrel	60
Beaver	3
White-footed Mouse	3
Cooper Mouse	2
Muskrat	1
Brown Rat	3
Rabbit	11
Elk	6
Deer	166
Horse	3

Birds

Trumpeter Swan	1
Ruffed Grouse	1
Prairie Chicken	2
Bobwhite	2
Domestic Chicken	5
Wild Turkey	6
Passenger Pigeon	29
Crow	1

Turtles

Box Turtle	73
Snapping Turtle	2

Frogs 6

There were also salamander bones found in the feces from a burial and identified by Dr. Case of the University of Michigan.

Here again the bones of the Deer are by far the most numerous, and it is interesting to note that the greatest number of bird bones are of the Passenger Pigeon. Other interesting bird remains include the Trumpeter Swan, and the Prairie Chicken.

Several species listed are without doubt introduced into the site in recent times. These are the various mice, the Horse and possibly the Flying Squirrel. The mice

and Squirrel may have been brought into the cave by owls, the bones coming from the pellets of these owls. The Chicken bones are of recent origin.

BOONE ROCK SHELTER AND CAMP SITE

This shelter was excavated by Dr. Wm. C. Mills in 1905 as was the camp site immediately in front of the shelter. In his report Mills (1912) mentions animal remains from the shelter as follows:

> "The animal bones usually expected to be present in the habitation sites of prehistoric man were few within the shelter, and no doubt many had been carried away by former searchers; however specimens of the bones of the Virginia deer, black bear, raccoon, and wild turkey were secured".

Mills lists the bones found in the camp site and all were found in the recent study except the Otter. Since all of the material carries Boone Rock Shelter accession number this material is listed together.

The animals identified from this site are as follows:

Mammals

Black Bear	10	Gray Squirrel	1
Raccoon	5	Beaver	3
Skunk	1	Domestic Pig	1
Cougar	1	Elk	24
Woodchuck	1	Deer	233
Birds			
Mallard Duck	1	Wild Turkey	9
Fish			
Catfish	2	Drum Fish	2
Mussels	17		

BUZZARD ROCK SHELTER

This shelter was also excavated by Dr. Wm. C. Mills in 1905 but very few bones were found in it. Only one bone of the Elk and three bones of the Deer were found.

TWINSBURG ROCK SHELTER

This shelter which is located in Summit county was excavated by Mr. Charles Shipman and others. The material was sent to the Ohio State Museum for study, in 1944, and the animal remains were identified by the writer.

The shelter contained considerable contact material such as old crockery fragments, broken clay pipes etc., confirming a report that a man lived in the cave in relatively recent times. The finds certainly bear this out, as do the animal bones, those of the Ox or Cow being found.

The following animals were identified from this shelter:

Mammals

Opossum	3	Woodrat	8
Big Brown Bat	13	Cooper Mouse	1
Black Bear	12	Meadow Mouse	2
Raccoon	67	Muskrat	4
Mink	1	Woodland Jumping Mouse	5
Wild Cat	6	Porcupine	18
Woodchuck	13	Rabbit	82
Chipmunk	2	Domestic Pig	7
Gray Squirrel	25	Deer	259
Beaver	97	Ox or Cow	13
Birds			
Wild Turkey	22	Barred Owl	1

Perhaps the most interesting find in this shelter was the remains of the Woodrat. This is a considerable extension of the known range of this mammal in Ohio, the only other records for northeastern Ohio being a report from Tuscarawas county,

Langdon (1881) and another from Ashland county, Kirtland (1838). Here again we find the most abundant mammal to be the Deer. Another interesting find is the Woodland Jumping Mouse. Those which I do not consider associated with the Indian occupation of the shelter are the Jumping Mouse, Big Brown Bat, Cow, Cooper Mouse, Meadow Mouse, Woodrat and possibly the Chipmunk. Here is also shown the comparative abundance of the Beaver and Porcupine, a condition that is often noted in the northern Ohio Indian village sites.

CANTER CAVES

These caves consist of two large rock shelters, one known as "Indian Cave" and the other known as "Echo Cave". They are situated about five miles northwest of Jackson in Jackson county, Ohio and were excavated by the owner Mr. George N. Miller in 1925. A report by Shetrone (1928) on the finds from these caves mentions the commoner species of animals found.

The bird bones from this site were sent to the National Museum for identification and reported in the Wilson Bulletin, Wetmore (1932). This report lists 19 species identified including the first report of the Mississippi Kite in Ohio. In a re-examination of material from these caves one other bird species was identified.

The animal bones from these caves are as follows:

Mammals			
Opossum	14	Chipmunk	1
Black Bear	11	Gray Squirrel	43
Raccoon	158	Flying Squirrel	2
Mink	2	Beaver	27
Skunk	72	Woodrat	4
Dog	6	Muskrat	3
Gray Fox	9	Porcupine	5
Gray Wolf	6	Rabbit	44
Wild Cat	13	Domestic Pig	1
Cougar	4	Elk	51
Woodchuck	25	Deer	344
Birds			
Mallard Duck	2	Prairie Chicken	1
Blue-winged Teal	7	Bobwhite	1
Wood Duck	4	Wild Turkey	51
Ring-necked Duck	2	Passenger Pigeon	30
Lesser Scaup Duck	5	Barred Owl	2
Swallow-tailed Kite	1	Flicker	1
Mississippi Kite	2	Pileated Woodpecker	2
Red-shouldered Hawk	1	Raven	2
Duck Hawk	1	Crow	1
Ruffed Grouse	9	Grackle	1
Turtles			
Box Turtle	52	Snapping Turtle	5
Mussel Shells	206		

In the above list it is again the deer that is the most abundant animal found. Of interest also among the mammals is the discovery of the Porcupine which has been found in only one other rock shelter in Ohio, the Twinsburg shelter. The Woodrat record is also of interest. Among the birds the best records are the Mississippi Kite, Passenger Pigeon, Raven and Prairie Chicken.

SUMMARY

This paper brings together information on animal remains secured from archaeological excavations of six rock shelter sites in Ohio. A total of 2845 bones were examined and 2620 specimens identified, representing 32 species of mammals, 23 of birds, 2 of turtles and 2 of fish. The deer was the most abundant mammal found in all of the shelters. Other mammals of some special interest are the Woodrat, Porcupine, and Woodland Jumping Mouse. In the list of birds the

Mississippi Kite represents the only known record of this species for Ohio; the Passenger Pigeon, found in three of the shelters, was the most abundant bird at in Kettle Hill shelter; the Raven was found in one shelter and the Prairie Chicken in two shelters. The remains of the Cow, Horse, Pig and Chicken are not surprising among these bones since the shelters were often visited by picnic parties and others in recent times. It may be noted that the shelters which have produced the largest number of bones are those that have been completely excavated, which includes Canter Caves and Twinsburg rock shelter; the others were only partially excavated.

ACKNOWLEDGMENTS

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In the preparation of this excellent book the author has very satisfactorily attained his objective, . . ."to bring home to the student and investigator an appreciation and understanding of how radioisotopes can fit into his program and then to show how the experimental work can be undertaken."

The basic principles of tracer methodology are discussed without being greatly complicated by an over treatment of nuclear physics. The characteristics of individual radioisotopes are discussed from the standpoint of kinds of investigations to which each is suited and information is provided which will help the investigator determine methods of assay, necessary facilities and amounts of activity to be used. Type experiments are outlined and discussed and this should prove helpful to an investigator planning a particular experimental approach to a biological problem.

Chapters on Autoradiography and Paper Chromatography are also included.

The cited references which assemble individual papers from widely scattered publications places an extensive bibliography in the field of radioisotope research at the hands of researchers. A glossary of selected terms in nuclear science is provided.

R. H. Bŏhning.

SEMANTIC ANALYSIS OF A FEW ANATOMICAL TERMS OF THE 18TH-CENTURY DELAWARE INDIANS[1]

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In continuance of his semantic studies in the aboriginal culture area of the 18th-century Delaware, this writer wishes to present a semantic analysis of a few representative anatomical terms. They derive from the same source which had yielded the Delaware tree names such as analyzed in last year's volume of The Ohio Journal of Science (Mahr, 1954). It is the Rev. David Zeisberger's Dictionary in Four Languages, compiled by him and a few other Moravian missioners in the Tuscarawas Mission area of Eastern Ohio, during the 1770's and 1780's. With no discrimination between the two main Delaware dialects, Unami and Munsee, the Delaware word material is listed in the fourth column of the Dictionary's 362 manuscript pages; the three others being, from left to right, English, German, and Onondaga, an Iroquoian dialect of Eastern New York State: Zeisberger (1887). This remarkable work will henceforth be cited, in these pages, as, 'the Dictionary'.

Although it is not intended to burden the reader with linguistic details, yet we cannot dispense with a reasonable minimum, which is inherent in the method, and which must be, and can be understood also by him who is not a linguist.

Whenever the Dictionary is directly quoted, Zeisberger's and other Moravians' spelling of Delaware words, which is based on their native German phonology, has been transcribed, in these pages, into a more intelligible form of writing, mainly based on English spelling usage. For example, *sch* has been changed into *sh*, *g* into *k*, *b* into *p*,-*eu* into-*ew*, *j* into *y;* while the vowels, *a*, *e*, *i*, *o*, *u*, also when duplicated for length, have retained their German sound values, the same applying to *ch* (as in Scottish, lo*ch*). Wherever *tch* occurs, it is the equivalent of *ch* (as in English, *ch*ur*ch*). Zeisberger's *wu-*, or *we-* (*w* plus sheva), is represented in these pages by *w'-;* analogously, *mu-*, or *me-*, by *m'-*. Where (') occurs, following any other consonant, or preceding a vowel, it has the sound value of a 'stop'.

These phonological approximations were adopted after a careful check against the sound conditions in Delaware dialects extant today, such as presented by Voegelin (1946).

As to our topic, it may be noted that, on the aboriginal level, the modern science of anatomy would have been entirely meaningless to the Delaware (or the other American Indians, for that matter); as devoid of sense or purpose as any of the descriptive or taxonomical studies of animals and plants, rated so highly by the White Man.

BODY (generic)

The Delaware Indian's awareness of his body was tantamount to an awareness of the functional mechanics of the body's parts. Nevertheless, in the Dictionary there occurs a term *hâk/ai*, defined as 'body', Germ. 'der Leib'. The stem *hak-*, likewise present in *hâk/ii*, 'land, ground, soil', evidently carried a basic connotation of 'plane', in the sense of 'two-dimensional extension'. This is made

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into something tangibly concrete by the noun-affix *-ai*, which gives it the approximate meaning of 'plane-bounded something', that is, 'a tri-dimensional body'. Primarily stereometrically determined, it assumes a *personal* significance when appearing pronominally prefixed as *n'hák/ai*, 'myself', or *k'hák/ai*, 'thyself'. Not any more, though, than the English term 'person', does Del. *hák/ai* carry a connotation of 'body (as organism)'.

BODY (as organism)

Yet for this latter concept there also occurs a name in the Dictionary, likewise defined as 'body', Germ. 'der Leib'. It is here given analytically rewritten so as to reveal its multiple-stem connotations: **wachtchu/'chew'/hée/pi*, a compound from *wachtchu-*, '(it is) full, bulky' (also defined as 'mountain, hill'); *-achêwi-*, an adjective denoting, 'strong, spirited'; *-hee'-*, a transitive-verb stem with the meaning of 'to make, to constitute (the thing indicated)'; and finally a noun-affix *-pi*, signifying 'matter, substance, material'. Hence the composite meaning of **wachtchu 'chew'/hée' pi*, for 'body (as organism)', appears to be: 'matter constituting spirited bulk'.

BODY TISSUES AND SUBSTANCES

Hunters as they were, the Delaware Indians as well as other American aborigines were in the habit of cutting animal bodies apart, thus growing familiar with the various tissues and related substances; and also with the fact that these were essentially the same in the body of Man. Hence the names for them, such as listed in the Dictionary, show no discrimination either.

SKIN

The fact that for 'skin' there occur two terms, *chai* and *chees*, is easily explained from their Dictionary definitions: *chai* is defined as 'skin', while *chees* in one place is called 'hide', Germ. 'die Haut', but in another is defined as 'leather'. This indicates that *chees* was used for 'processed skin', as a market term in the Indian-White skin trade. It appears however that *chai* likewise could apply to 'skin (as a utility)', as long as the skin's provenience from a living animal was still in evidence; as for instance in *ask/chái*, 'a fresh skin' (*askiwi* meaning 'raw'). As a word formation, **'ch/ái* most likely is shortened from **ach ái*, in the Unami dialect, or **wochk ái*, in the Munsee or Early Unami. The prefix *ach-* (**wochk-*) signifies '(existing) on the top, or, on the surface'. The noun-affix *-ai*, which follows it, invariably indicates 'concrete manifestation of (phenomenon mentioned)'. Hence the composite meaning of **'ch/ái* is: 'substance existing on the surface'.

In the other term, **'ch hées* (Dict., *chees*), the prefix *ach* (**wochk-*), '(existing) on the surface', precedes a participle affix, *-hees*, signifying: '(animate) object, or person, habitually making, or doing, (the thing indicated)'. Hence the basic meaning of **'ch, hées* is 'that which habitually makes (constitutes) something existing on the surface'.

A pertinent remark may here be in order because of its wider applicability. Doubtless it was the mere difference in the sound of their slightly divergent semantic components, which kept these two terms from being applied synonymously; for it is most likely that, among the many people who used them day in and day out, not a single one was still conscious of their basic meaning, at a time when they had long become fixedly associated, throughout the entire Delaware nation, with definite objects of definite properties. This is true not only for these two names for 'skin' but for practically all the terms analyzed in these pages; with the possible exception of the one or other in which the basic meaning may have persisted so near below the surface of the accepted usage that the popular mind had remained aware of it.

MUSCLE

What is 'muscle' to the anatomist, is 'flesh' in common parlance, and 'meat' to the hunter. In the Dictionary, all three of these are covered by the term **'o/yóos*, defined in one place as 'flesh', and in another, as 'meat'. Evidently it is connected, both etymologically and semantically, with Del. **w'yáw'/ew*, '(being) good, strong, efficient'. This connection is confirmed by an early-17th-century Virginia-Indian listing, *wiaaws* (**w'yáaws*), with the definition, "the Leane of any Flesh"; Strachey (1612), Sheet 9. Hence, a basic form **w'yáw'/s*, for **'o/yóos*, appears indicated. It is compounded with the initial stem **w'yawch-*, which possessed a comprehensive meaning of 'desirable, by virtue of his (or its) superior qualities'. It is followed by the inanimate-noun affix *-s*. Thus the basic meaning of this name for both 'flesh' and 'meat' is '(desirable) quality-matter'.

BONE

In their name for 'bone', *wochk/án*, the Delaware expressed no concern with the structural function of 'bone' as support of the animal body, but the term *wochk/án*, apart from its use as 'bone (generic)', rather applies specifically to such bone only, as forms the surface confines of body cavities, the thoracic one, for instance. The stem, *wochk-* denotes '(existing) on the surface (of)'. Combined with noun-affix *-an*, 'substance, matter, thing', the composite meaning is 'surface matter' or 'surface thing'. For this reason, none of the Delaware names for skeleton bones of the extremities is formed with *wochk/án* as a component, each having its own appellation indicative of a specific property.

TENDON

Another body tissue significant to the Indian was the 'tendon', which was found to be connected with both flesh and bone and therefore instrumental in all outer movements of the body. Under the heading 'sinew', Germ. 'Flechse, Sehne', the Dictionary carries the following terms for 'tendon': **(w)'tch/hêet'*, or, **(w)'chsh'/hêet*, a variant, probably Unami, of the former. Another term is **hât/hees*. The two first-mentioned are compounded with a stem **(w)'tch'kw-* (or Unami **(w)'sh'k-*) denoting '(being in) motion'; followed by a participle affix, *-heet*, indicating that '(it, at this moment is,) making, or doing (the thing mentioned)'; the composite meaning being: 'that which is making (bringing about) motion'.

The other term for 'tendon', **hât/hees*, is formed with a verb stem, *hat-*, potentially meaning, depending on the formative, either 'to be there *passively*', or 'to be there *as an agent*'. Here evidently meaning 'to be there as an agent, to function', *hat-* is combined with the participle affix, *-hees*, which signifies that a '(person or animate thing, is habitually) making, or doing (the thing indicated)'; *comp.*, Voegelin (1946), page 148, §84:9. The composite meaning is: 'that which habitually makes (causes to) function'.

These analyses clearly show that the Delaware names for 'tendon' really define the function of a nerve, rather than of a 'tendon'. This is not at all surprising in an aboriginal culture area in the 18th century, if one considers that even today popular usage in most European languages, including English and German, fails to differentiate between 'tendons' and 'nerves'. This misconception has been persisting despite the fact that not only the nature of the nerves but even the functional difference between motor and sensory ones had been discovered as early as about 300 B. C. by Herophilos of Chalcedon, a physician of the Alexandrian school of medicine. Yet the fact remains that not even Herophilos himself, though rightfully regarded as "the father of anatomy", had arrived at a clear separation of nerves from tendons; Singer (1941), page 199b.

BLOOD

Being hunters and warriors, the Delaware naturally were familiar with 'blood' and its being encountered everywhere in the animal body, running in 'blood vessels', which spill it when cut or torn. They knew that too heavy a loss of blood resulted in death, yet they had no knowledge of its physiological function. 'Blood' to them was no more than a 'red animal-substance'. This is indicated in the basic meaning of their name for 'blood', *móok/um*, which according to the Dictionary meant both 'blood' and 'blood vessel'; while the German definitions of one of the entries, 'Blut' and 'Geblüte', show that in addition *móok/um* also signified 'the mass of blood in a living animal, or human, body'.

Evidently *móok/um* is a contraction, probably Unami, of **m'hóchk/'m*. Its initial component is a stem **m'hochk-*, often occurring as *machk-*, and denoting 'red' or 'reddish brown'. It precedes the noun affix *-'m* (*-um*, *-am*), the most common meaning of which is 'animal, or vegetal, substance' respectively. This adds up to the composite meaning of 'red animal-substance'.

FAT

This non-initial noun-affix, *-'m* (*-um*, *-am*) indicating 'animal substance' is likewise present in one of the Dictionary's two names for 'fat', **po/'m/ti*, which appears to be compounded from a stem *po-* denoting 'swelled, bulging', as for instance in *póa/wi*, 'pregnant'. It precedes the noun affix *-'m*, 'animal-substance'; which is followed by the place-time adverbial affix *-ii* (*-ee*) with its dual connotation of 'now-and-here'. This gives **po/'m/ti* a basic meaning of 'bulging animal-substance (existing) now-and-here'.

The Dictionary's other term for 'fat', spelled *wikul*, is also listed in Brinton's Lenape-English Dictionary (1889), with the definition 'fat in an animal's belly'. This limiting addition, 'in an animal's belly', prompts an analytical rewriting, **wíichk'w/ool*, compounded from **wíichk/ew*, '(it is) enveloping'; and an inanimate-plural verb suffix, *-ool*, indicating that 'they have completed (the process mentioned)'. Thus the basic meaning of **wíichk'w/ool* is: 'they have completed enveloping'; or more explicitly: 'they (that is, the pieces of fat) thoroughly envelop (things in an animal's belly)'.

It is semantically significant that the Dictionary lists these two terms for 'fat (as an animal-body substance)' also with the definition 'tallow', Germ. 'Unschlitt', that is, 'fat (as a utility)'; while under the heading 'grease', Germ. 'Fett, Schmeer', there occurs **shaach/'m/êw*. It is compounded from a stem *shaach-*, meaning 'slippery'; *-'m-*, 'animal-substance'; and 3rd-pers.-sing. verb-suffix, *-ew*, 'it is'. Composite meaning: '(it is) a slippery animal-substance'. The Dictionary even goes one semantic step farther, right into the accultural domain: under the heading 'oil', that is, the White Man's vegetable oil, it not only lists **shaach/'m/êw* but also **po'm/ti*, expressly adding the definitions 'oil, fat, tallow'. This indicates that about 1782, when this entry was made, the two terms, for all practical purposes, had become synonyms.

BILE

With no inkling of its physiology, yet as consumers of game animals all the more wary of its bitter taste, the Delaware hunters were well acquainted with 'bile'. Under the heading, 'gall', Germ. 'die Galle', the Dictionary carries two terms: **wti's/'m/ii* and **wti's/'w/ii*. Doubtless the initial component of both is a stem, *wii's-*, 'bitter (of taste)'. In **wti's/'m/ii* it is combined with *-'m*, 'animal-substance'; and place-time affix *-ii*, 'now-and-here'. The composite meaning is 'now-and-here (exists) bitter-tasting animal-substance.'.

The other name, **wti's/'w/ii*, with no reference to 'animal-substance' is directly formed with an adjective **wti's/'wi*, 'bitter (of taste)', again affixed with *-ii*,

'now-and-here'. Its basic meaning is '(something) bitter-tasting now-and-here'. In analogy with Shawn. *wiith/wi*, which is defined as both 'bile' and 'gall-bladder' (Voegelin, 1938-40), the same may apply to Delaware, which moreover has previously been shown to possess *one* name, **m'hóchk/'m*, for both 'blood' and 'blood vessel'.

BRAIN

As the Delaware name for 'brain' the Dictionary lists, *tump* (spelled: *tumb*). This term (as do other Delaware names for body parts) never occurs without a possessive prefix, mostly the indefinite possessive, *w'-*. According to an inviolable Delaware speech habit, this *w'-* (or any other possessive particle) could not be prefixed to a stem beginning with a vowel, without the intrusive consonant *-t-* between *w'-* and the initial stem vowel. Hence, *tump*, as listed in the Dictionary, must be analytically rewritten, **wt/uump*. The stem, **-uump*, following the possessive unit, *wt-*, is a (Unami) dialect form of **w'mp*, which denotes something like, 'contained inside-matter'. This meaning may be inferred from the presence of **w'mp*, with vowel implement, *-ii-*: in the Dictionary, as **wiimp* (spelled, *wimb*), defined as, 'heart of a tree'; and, again as **wiimp* (spelled, *weimb*), defined as 'the marrow of a bone', in Strachey's vocabulary of the Virginia Indians (who were Southeast-Algonquians, if not even Delaware), which he compiled, during the first decade of the 17th century, while living among them as a companion of Capt. John Smith, of Pocahontas fame. On the basis of this dual occurrence of **wiimp* and its definitions here quoted, it appears justified to ascribe to **wt/uump*, 'brain', the primary meaning of, '(somebody's) contained inside-matter', or even, '(somebody's) marrow'. This seems all the more plausible since 'bone marrow' and 'brain matter' are so similar in both their appearance and consistency that those aboriginal hunters could easily have regarded them as one and the same substance.

Substance, and no more; for the Delaware term carries no connotation whatsoever of 'thinking' or 'reasoning'; nor did there, or even *could* there, exist an *associative* link between 'brain' and 'mind'. Why not, we will shortly see.

HEAD

This naturally leads to a discussion of 'the head', the seat of the brain. It is significant that the Delaware and the Shawnee possessed an identical name for 'head', listed in the Dictionary as **w'iil*. This appellation is strongly evaluating, due to the fact that, again in both Delaware and Shawnee, **w'iil-*, as a prefix in compounds, also denotes 'superior qualities', and specifically 'potent virility; valor', when applied to a male, animal or human.

In Shawnee however, but not in Delaware (or, maybe, *no longer* in Delaware) there exists another term for 'head', which is *-tépee* and occurs in compounds only; with an almost identical parallel form, *(-)tépi*, defined as 'brain', and never appearing without a possessive prefix. Its basis is a primary stem, **t'p-*, apparently of considerable antiquity in that it joins two elemental roots. The first, *t'-*, is a primary demonstrative particle expressing 'here, there (is)', and necessarily followed by another element indicating *what* there is. In the case of *t'p-*, this implement is an equally elemental root *-'p*, denoting 'position on top' or 'upward tendency'. Thus the basic meaning underlying the Shawnee term for both 'head' and 'brain' is 'here, there (it is) on top'. Most likely, at an earlier stage of their language the Delaware too used **tép/ii* (or a similar compound) as their name for 'head', which in Zeisberger's days (about 1780) had ceded to that evaluating term **w'iil* with its connotation of 'superior qualities'. That this is what happened may indirectly inferred from Strachey's Virginia-Indian (Delaware) vocabulary of about 1612, which lists a name for 'head', *mmtabukham*, whose semantic nucleus likewise is that stem *-t'p-*, stressing the head's 'position on top'; Strachey

(1612), Sheet 10. Analytically rewritten, Strachey's Virginia-Delaware form presents itself as **m'm'/täp/ûchk/'äm*, which in (Zeisberger's) Eastern Delaware, if a parallel term had existed, might have appeared as **w'm'/t'p/ûchkw/'m*. The first component, **w'm'-*, most likely is an unaccented form of **wémi-* (Virg.-Del. **mémi-*?), a superlative-prefix denoting 'all, most'; *-t'p-* is that semantic nucleus, meaning 'on top'; *-uchkw-* is a stem signifying 'solid body' or 'solid object'; and *-'m* indicates 'body substance, body part'. This adds up to a basic meaning 'topmost solid body-part'.

May it be noted that this primary stem *t'p* may constitute a connecting link, be it ever so small, between the East-Algonquian and the Central-American language-and-culture areas, since its relation with Nahuatlan (Aztec) *-tépe·tl*, 'mountain top, hill', can hardly be doubted.

The high evaluation of the 'head', such as implied in the name *w'iil*, cannot be due to the fact that the main nerve center, the brain, is located in the head, for it is evident that those Indians, *aboriginally*, had no notion of these brain functions. True, they knew that brain injuries usually were fatal; and that therefore the surest and quickest way of disposing of an enemy was to drive a tomahawk into his head, or to mash it with a war-club. Yet, of the deeper reason for this they had no inkling. The brain, to them, was just a body substance like any other, which did not rate a special evaluating name as did the head. It did not occur to them that this brain matter was the place where their thoughts, opinions, resolutions were hatched. Popular belief in wide areas of the earth regards the heart as the seat of human emotions. The Delaware and Shawnee, as well as other American Indians, not only shared this belief but they also regarded the heart as the cradle of rational thought, as we will presently demonstrate.

HEART AND THINKING

Prefixed, as so many names for body parts, with the indefinite possessive *w'-*, the Delaware term for 'heart', listed in the Dictionary, is **w't'éeh'*. Similar, if not even identical, parallels occur in the related East-Algonquian dialects such as Shawnee, Miami, Mohican, etc. Likewise present in Delaware, as well as in those other languages, is a stem *-t'h'-*, defined as 'to think', whose *accented* form is *(-)t'éeh'*, defined as 'heart'. This basic and ancient stem **t'/'h'* seems to be a combination of the elemental demonstrative **t'-*, 'here, there (it) is', with *-'h'*, an equally elemental root-stem probably indicating '(involuntary) movement of body-organ'. This gives **t'_'h'* a basic meaning of 'here is movement'; obviously referring to 'the heart-beat'.

Further, the Dictionary lists a verb **w'teli/t'éh'/en* with a definition 'to think', but basically meaning 'one's heart to be thus (inclined)'. Here the element *-t'éh-*, signifying 'heart', is preceded by *w'teli-*, an adverbial prefix denoting 'thus; in this particular manner', and followed by the verb-affix *-en*. While in this compound the association with 'heart' is inevitable, it is more than doubtful whether as late as the 1780's when the current meanings for *-t'eh'-*, 'heart' and 'thinking', had been entered in the Dictionary, the *primary* association with the '*beat* of the heart' was still alive in anyone's mind.

HEAD AND "SENSES"

This should make it evident why it was hardly in honor of the 'brain' that the Delaware coined that evaluating name for 'head': *w'iil*, with its emphasis on 'superior qualities'.

Yet, not even on the most primitive aboriginal level will it be surprising to find the 'head' being rated 'superior'; for the simple reason that the human animal cannot have been long to discover his head to be the seat of four of those so-called "senses", on which he incessantly relies: sight, hearing, smell, and taste; while only the fifth, touch, extends all over the body.

SIGHT

The Dictionary lists a term **(w)'sh'k/ĭi/'nk* in three different places, under three different headings: (1) 'eyeball', (2) 'eye', and (3) 'face'. The common denominator in these three definitions is 'movement'. This is evident from the fact that **(w)'sh'k/ĭi/'nk* is based on an intransitive-verb stem, **(-)'sh'k-*, 'to be in motion'. This gives the composite noun (really a participle) the basic meaning of '(thing) being in motion now-and-here (*-ii-*)'. This 'being in motion' most of the time was observed as the truly distinguishing feature of the 'eye', particularly the 'eyeball', and thereby of the human 'face', and led to the coining of that one name for all three of them. Considering the paramount importance of the eye for the hunter-warrior, one is surprised to find in this Delaware term for 'eye' no connotation whatsoever of 'sight' or 'vision'.

Among the various verbs defined in the Dictionary as 'to see', 'to look, 'to gaze', etc., the intransitive **wáap/iin* clearly is the one which means 'to see (as function of the eye)'. It is based on an East-Algonquian stem *waap-*, primarily signifying 'light, bright, white, shining, etc.'; Voegelin (1938–40), pages 411–412; the final *-iin* is an intransitive-verb affix indicating 'to be (in said condition)'. Thus **wáap/iin* basically means 'to be in a condition of light, brightness'. Evidently the idea behind it was that the eye, although *not the source* of light, yet *made it to be light* for its possessor. Goethe, the German poet, expressed a similar thought in these two lines: Wär' nicht das Auge sonnenhaft,/die Sonne könnt' es nicht erblicken" (in translation: "Were not of sun the eye possessed,/the sun it never could perceive").

It may be noted that the Delaware term for 'being blind' metaphorically, and maybe even euphemistically, stated that the afflicted person was 'blindfolded'. The term for 'being deaf', by the way, is a similar understatement, implying that the deaf person 'by and by is thoroughly stopped up'.

HEARING

The organ of hearing, 'the ear', is named in Delaware **w'hítt/ew/achkw*, which usually occurs in the plural. As so many other names for body parts, it is not used without a possessive prefix, here *w'-*. This precedes a stem *hitt-*, which primarily indicates 'spreading apart' (as in *m'hítt/ukw*, 'a tree', and **m'hitt/achp/-oákan*, 'childbirth'). It is combined with 3rd-pers.-sing. verb-suffix *-ew*, 'it is, it does (the thing indicated)'; which is followed by a non-initial noun-stem *-'chkw* (*-uchkw*, *-achk*, *-aak*), basically meaning, 'wood, tree, solid object (in compounds)'; Bloomfield (1946), page 105, §60. This adds up to the composite meaning '(somebody's) solid object, it spreads away', that is, from the side of the head.

There is no Delaware intransitive verb for 'to hear (as function of the ear)'. The transitive one which comes closest to it is **ach/kĭin/chen*, listed in the Dictionary under the general heading 'to hear'. Its composite meaning approximately is 'to receive-by-ear (*-ch/en*), very sharply (*ach/kiin-*)', and it is necessarily to be followed by a grammatical object. The Dictionary's definition is 'to hear well'.

A close second, likewise occurring under the heading 'to hear', is **pen/t/ámen*. Its initial component is **pen/t* (properly, **pem/t*), a combination of a stem *pem-* denoting 'movement onward, or toward', and *-t-*, instrumental affix implying 'by ear'. This is followed by the transitivizing verb-affix *-amen*, 'to carry out (action indicated)'. Hence, the basic meaning of **pen/t/ámen* is 'to carry out action of moving toward (an object) by ear'; that is, 'to really hear it'.

SMELL

For the organ of smell, 'the nose', a Delaware name, **w'kéw/an*, is listed, which in another place of the Dictionary is defined as 'beak (of a bird)'. From

Shawnee parallels it becomes apparent that in both dialects the term denoting both 'nose' and 'beak' was based on East-Algonquian **k'w'*, a stem indicating 'projecting freely (from basis); being unattached'; Voegelin (1938–40), pages 308–309. Prefixed with the indefinite-possessive *w'*-, and followed by the noun affix *-an*, **w'kéw'an*, 'nose, beak', reveals a basic meaning of '(indefinitely possessed) freely-projecting thing'.

For the personal-transitive verb 'to smell (something)' the Dictionary carries *mel áam*, a verb in which the transitivizer *-aam* is preceded by a stem *mel-* (*m'l-*). Since evidently it is likewise present in Shawn. *mool-*, 'awareness of danger, of future happenings' (Voegelin (1938–1940), page 366), the accepted meaning of Del. *mel-*, 'smelling; perceiving by nose', may well represent a very ancient meaning elementally lodged in East-Algonquian **m'l*, 'getting the scent of something', occasionally as a foreboding of impending danger.

Here is the reason why this stem *m'l* appears almost primeval: being a direct opposite to the primary root *-'p'*- 'position on top, upward tendency' (*cf.*, above, page 367), *-'m'*- indicates 'position below, downward tendency'; note that **'p/uchkw*, basically meaning 'solid thing (*-'chkw*) tending upward (*'p-*)', signifies 'a rock, cliff', while **'m/úchkw*, 'solid thing tending downward (*'m-*), is the Delaware name for 'beaver' (going down into the water). In *m'l*, this root *-'m-*, 'tending downward', combines with another primary root, *-'l-*, evidently an instrumental indicating that something is done by 'breathing'; note: **'l'/éh/heen* (or, with emphatic stem reduplication, **'l'h/'l'éh/heen*) 'to draw breath' (*cf.*, above, page 368: (-)*t'éeh'*, 'heart'). This gives *m'l-* a basic meaning of 'tending downward breathing', which perfectly describes an animal, such as a dog, picking up the 'scent' of something 'by sniffing' the ground.

May it be remarked that the shift of meaning, in Shawnee, from 'scenting (by nose)' to 'becoming aware (of danger, or future events)' is far from being limited to Shawnee but rather is a wide-spread semantic phenomenon. The English-speaking nations 'scent a plot'; the Germans say 'Gefahr wittern' ('to scent danger'); the French speak of 'avoir vent de quelquechose', which literally means 'to have the wind of something', 'le vent' being 'the wind' which carries 'the scent'.

TASTE

The Delaware knew the surface of the tongue to be the seat of their "sense" of 'taste'. Sufficient evidence of this is their personal-transitive verb **noochkwá t/-amen*, defined in the Dictionary as 'to lick with the tongue'. In its basic meaning, though, this Delaware verb possesses no connotation whatsoever of either 'licking' or 'tongue', but rather is formed with a personal-transitive verb-stem **noochkw-*, which indicates 'examining, investigating'. Semantic analysis of its remaining components shows that **noochkwá t amen*, 'to lick with the tongue', basically means 'person to cause (*-t/amen*) the examining (of something)'. Nothing is said about 'the tongue' being caused to do the examining.

Neither does the Delaware name for 'tongue', **w'íil'a'no* carry a connotation of 'taste'. Except for their initial component, the prefix *w'iil-*, 'of superior quality', neither Del. **w'íil'a'no* nor its Shawnee parallel **w'íil a'ni* can be analyzed with certainty. It is not unlikely, though, that there exists a connection with Shawn. *w'iilenwi*, meaning 'fat part of meat' (that is, 'superior meat'), which even may be a more basic, and less specific, form of Shawn. **w'íil'ani*, 'tongue'; the same applying, analogously, to Del. **w'íil a'no*, 'tongue', with a conjectural basic meaning of '(thing) being of the nature of superior meat', in brief, 'being fleshy'.

With no semantic relation to 'tongue', two transitive verbs occur in the Dictionary, defined as 'to taste (something)': *kut ánt amen*, and *mesh ánt/amen*. Both employ the formative complex *-ant amen* meaning 'person acting (*-ant-*) to carry out (*-amen*) (the action indicated)'. In the one, this formative complex

is preceded by a verb-stem **ku't-*, 'to swallow'; in the other, by a verb-stem *mesh-* denoting 'to stop, halt, impede'. It amounts to this: 'to taste something' according to the one verb, is tantamount to 'swallowing' it. According to the other, it is identical with 'to suspend the swallowing of it' long enough to get its 'taste'.

In analogy to the other categories of sensory perception previously examined, the Dictionary lists numerous verbs indicating diverse aspects of 'taste'; specifically, 'taste sensations' evoked by various substances. Such verbs, for instance, as 'to taste pleasant', or 'unpleasant', 'sweet', 'sour', 'bitter', etc. Most of these are formed with stems entirely unrelated to each other, and since they have no bearing on anatomical terminology they will not be discussed in these pages.

SUMMARY

This semantic analysis of Delaware anatomical terms may be summed up in the following statements: (1) in Zeisberger's days, each of these aboriginal terms possessed a popular meaning, accepted and known as the name of a body tissue, body substance, etc. Semantically, they are *secondary* meanings which correspond with the definitions of the terms in the Dictionary; (2) by an analysis of its Delaware multiple-stem equivalent, each of these *secondary* meanings can be traced to its basic significance, which is revealed by the composite meaning of its *primary* constituents; (3) while all of these basic meanings are descriptive, some also are evaluating, others stress the consistency of a body substance, others emphasize the dynamic function, or the mere motion, or some other striking features, of inner or outer parts of the body; (4) with a few obvious exceptions, these basic meanings are most unlikely to have survived in the consciousness of the 18th-century Delaware, from whose lips Zeisberger and his collaborators had gathered this anatomical terminology and its current meanings, such as recorded in the definitions of the Dictionary.

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THE ESTIMATION OF THE FREQUENCY OF A RECESSIVE SEX-LINKED GENE BY THE METHOD OF MAXIMUM LIKELIHOOD

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Neel and Schull in their recent book *Human Heredity* (1954) consider the problem of the estimation of genetic parameters by the method of maximum likelihood. The reader is referred to their book for a brief clear discussion of the theory behind this method and its advantages. Considering the case of the estimation of the frequency of a recessive sex-linked gene, Neel and Schull derive the following formula wherein n_{dd} stands for the observed number of females with the recessive phenotype, n_{DY} for the number of males with the dominant phenotype, n_{dY} for the number of males with the recessive phenotype, N_f for the total number of females observed, and N_m for the total number of males observed:

$$q^* = \frac{-n_{DY} + \sqrt{n_{DY}^2 + 4(2N_f + N_m)(2n_{dd} + n_{dY})}}{2(2N_f + N_m)}$$

It is the purpose of this paper to describe an alternative solution of the problem which yields an equivalent formula. The variance of the formula will also be calculated. These formulae may be considered to have an advantage over those derived by Neel and Schull in that they can be reduced to simpler forms when the ratio of males to females is taken to be unity. The question as to how far from unity the sex ratio may deviate and yet for practical purposes be regarded as unity in the carrying out of computations will be considered later on in this paper.

DERIVATION OF THE FORMULA FOR THE MAXIMUM LIKELIHOOD ESTIMATE OF THE FREQUENCY OF A RECESSIVE SEX-LINKED GENE

Let N stand for the observed number of females in the population. Let x stand for the sex ratio, viz., the number of males divided by the number of females. Then the number of males can be represented by xN, and N plus xN will be the number of individuals in the total population. Let k stand for the proportion among females of females with the character. Let g stand for the proportion among males of males with the trait. Let q stand for the true frequency of the recessive sex-linked gene a, a numerical value of which is to be estimated from the available data. In Table 1 the population is divided into four classes according to genotypes, Aa and AA among females being grouped since these are phenotypically indistinguishable. The relative frequency, the numerical frequency, and the probability of occurrence of each class are also given.

The exact probability (P) of getting the observed sample can be expressed as:

$$P = \frac{(N+xN)!}{Nk!\quad N(1-k)!\quad xNg!\quad xN(1-g)!} \cdot (q^2)^{Nk} \cdot (1-q^2)^{N(1-k)} \cdot q^{xNg} \cdot (1-q)^{xN(1-g)}$$

The specific problem here is to derive an expression for q such that for this value of q the probability of getting the observed sample is maximum. A solution giving a maximum logarithm of the probability will be identical with the desired solution. Let C stand for the first factor in the right-hand member of the equality.

Then, taking natural logarithms of both sides of the equality, we have:

$$\log P = \log C + 2Nk \log q + N(1-k) \log (1-q^2) + xNg \log q + xN(1-g) \log (1-q)$$

The next step is to take derivatives of both sides of the equality with respect to q:

$$\frac{d \log P}{dq} = \frac{2Nk}{q} - \frac{2N(1-k)q}{(1-q^2)} + \frac{xNg}{q} - \frac{xN(1-g)}{1-q}$$

The third step is to set the derivative equal to zero and to solve for q. When the derivative has zero as its value, the estimated value of q will give the maximum value for the probability of getting the observed sample, and is the value of q desired. This maximum likelihood estimate of the parameter q may be designated as above by q^*, and is:

$$q^* = \frac{-x(1-g) + \sqrt{x^2(1-g)^2 + 4(2+x)\quad(2k+xg)}}{4+2x}$$

By making the appropriate substitutions, it can be shown easily that the formula derived by Neel and Schull can be reduced to this formula. In the form derived here, the formula admits of a simplification when the sex ratio, x, is unity. In this special case, the formula reduces to:

$$q^* = \frac{1}{6}\left(g - 1 + \sqrt{1 + 10g + g^2 + 24k}\right)$$

TABLE 1

The Relative Frequency, the Numerical Frequency, and the Probability of Occurrence of Four Classes Resulting from a Recessive Sex-Linked Gene

Class	Relative Frequency	Numerical Frequency	Probability
aa	k	Nk	q^2
$A-$	$1-k$	$N(1-k)$	$1-q^2$
*a*Y	g	xNg	q
*A*Y	$1-g$	$xN(1-g)$	$1-q$

VARIANCE OF THE ESTIMATE

The variance of the maximum likelihood estimate can be found by use of the following formula (Hogben, 1946):

$$-\frac{1}{\sigma^2_{q^*}} = \Sigma\left(n f_i \frac{d^2 \log f_i}{dq^2}\right)$$

Table 2 contains the quantities needed to calculate the variance. Applying Hogben's formula, we have:

$$\sigma^2_{q^*} = \frac{q(1-q^2)}{N(4q+x+xq)}$$

When x is unity, the formula for the variance reduces to:

$$\sigma^2_{q^*} = \frac{q(1-q^2)}{N(1+5q)}$$

TABLE 2

Theoretical Probabilities and Derived Quantities Needed for the Calculation of the Variance

Class i	Probability f_i	$\frac{d^2 \log f_i}{dq^2}$	$n f_i \frac{d^2 \log f_i}{dq^2}$
1: *aa*	q^2	$\frac{-2}{q^2}$	$-2N$
2: *A*−	$1-q^2$	$\frac{-2(1+q^2)}{(1-q^2)^2}$	$\frac{-2N(1+q^2)}{(1-q^2)}$
3: *a*Y	q	$\frac{-1}{q^2}$	$\frac{-xN}{q}$
4: *A*Y	$1-q$	$\frac{-1}{(1-q)^2}$	$\frac{-xN}{(1-q)}$
Total	—	—	$\frac{-N(4q+x+xq)}{q(1-q^2)}$

AN EXAMPLE OF ESTIMATION

Waaler (1926) studied the inheritance of several kinds of color blindness in the human population of Oslo, Norway. In the course of his investigation, he recorded the color vision of 9049 boys and 9072 girls. Among these he found 725 color blind boys and 40 color blind girls. The quantities needed for the application of the formulas derived in this paper are x, the sex ratio, which is here 0.9975; g, the proportion of males having the trait, here 0.08012; k, the proportion of females with the trait, here 0.00441. Substituting these values in the formula for q^*, we calculate that q^* equals 0.0772. Neel and Schull after applying their formula to the same data arrived at the same result. Using the formula for the variance derived above, we get 0.000006112 as the variance of q^*. Neel and Schull do not explicitly give a formula for the variance of their estimate of q, but they give the formula for the invariance. The formula for the variance is the reciprocal of the formula for the invariance. It may be noted here in passing that the formula they give for the variance of q estimated for the case of allelic sex-linked genes lacking dominance can not be used in the present case of a recessive sex-linked gene. Returning to Waaler's data, and recalling that the standard deviation is the square root of the variance, we get 0.00247 as the standard deviation of q^*. This is identical with the value given for the same quantity by Neel and Schull.

USE OF THE SIMPLIFIED FORMULAS WHEN THE SEX RATIO DEVIATES FROM UNITY

The question arises as to how far from unity the sex ratio may be and yet be regarded as unity in calculating the maximum likelihood estimates of q and its variance, and thus permit the use of the simplified formulas given above in which the sex ratio, x, does not occur. The answer to this question will depend on how accurately one wishes or needs to know the value of q^* and its variance. Concrete examples may help one to decide in a particular case. Let us assume that the proportion of males with the recessive sex-linked trait (g) is 0.2 and the proportion

of females with it (k) is 0.01. With these values constant, let x, the sex ratio, vary from unity to 0.9 to 0.8 to 0.7 to 0.6 to 0.5. The corresponding values of q^* by the long formula will be 0.168, 0.166, 0.164, 0.161, 0.157, and 0.154. The value of q^* by the simplified formula which assumes the value of x to be unity remains 0.168. It may be concluded that use of the simplified formula when x ranges between 0.9 and unity gives a value close enough to the true maximum likelihood value to serve as a useful approximation. Of course, the closer x actually is to unity, the closer will be the approximation. Thus, using the simplified formula for Waaler's data, taking x to be unity and rounding off g to 0.08 and k to 0.0044, we get q^* to be 0.0772. This is the same value we got when using the more exact figures given above in the long formula, x being 0.9975.

It may be noted here that whenever k is equal to the square of g, the maximum likelihood estimate of q is always equal to g, no matter what value x has. This fact can be easily verified by substituting various values for x in the formula derived in this paper, but always keeping k equal to the square of g. It follows that in this special case the formula for the maximum likelihood estimate of q can be simplified to: q^* equals g.

A few words may suffice on the use of the simplified formula for the variance. Supposing N (the total number of females) to be 1000, g to be 0.2, k to be 0.01, and x to be 0.9, q^* by the long formula is 0.166. The variance by the long formula is 0.000094 while by the simplified formula which assumes that x is unity, the value is 0.000088. The difference is 0.000006. The corresponding standard deviations are 0.0097 and 0.0094, differing by 0.0003. It may be concluded again that when x ranges between 0.9 and unity, the simplified formula for the variance can give a useful approximation to the exact maximum likelihood estimate. Thus using the simplified formula for Waaler's data, taking x to be unity, N to be 9072, and q^* to be 0.0772, we calculate the variance as 0.000006099. This value differs by 0.000000013 from the exact figure given above using the long formula with x as 0.9975.

SUMMARY

This paper describes solutions of the problems of deriving the maximum likelihood estimate of the frequency of a recessive sex-linked gene and its variance. The formulas derived are equivalent to those derived by Neel and Schull, but admit of simplification when the sex ratio is unity. The question is discussed as to how far from unity the sex ratio may deviate and yet be regarded as unity in computations.

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In Memoriam

PROFESSOR RAYMOND CARROLL OSBURN

1872–1955

Dr. Osburn was born January 4, 1872 on a farm in Licking County near Newark, Ohio. Due to certain physical handicaps he was deprived of much of the formal school experiences. In these early formative years his mother taught him, giving him the necessary background from which to ascend to heights of understanding through prodigious reading and his infatuation for the facts of nature that he gleaned from woods and fields of the countryside in which he spent his youth. With this uncommon substitute for formal training he entered The Ohio State University as a special student at the age of 23. It was not long, however, before he earned for himself regular academic standing and was given the Bachelor of Science degree in 1898. After graduation he taught classes at Starling Medical College and gained the Master of Science degree from his Alma Mater. With this he went to Fargo College, North Dakota, and then to Columbia University to earn his Doctor of Philosophy degree in 1906. While at Fargo he met Miss Mary Hubbell one of his students whom he later married on August 19, 1903.

He also held such positions as Assistant Professor at Barnard College of Columbia University, Professor and head of the Biology Department of the Connecticut College for Women, Scientific Investigator for the U. S. Bureau of Fisheries, and Associate director of the New York Aquarium.

When Professor Herbert Osborn retired from the chairmanship of the Department of Zoology and Entomology the university looked about for an outstanding candidate for the place. It was Raymond Osburn whom they chose to hold this post for 25 years. This was a period of rapid growth for the University and splendid expansion of the Department under his guidance.

For 20 years he guided the destinies of the Franz Theodore Stone Laboratory at Put-in-Bay, Ohio, and largely through his efforts the University acquired possession of Gibralter Island with the generosity of Mr. Julius Stone. Dr. Osburn was a member of the national honorary Society of Sigma Xi and held membership in 15 scientific societies. Early in his career he was sent by the American Museum of Natural History to study the aquatic life of the Caribbean Sea. Bryozoa, fishes and insects were his chief groups of interest on which he published many papers. Such was his repute that after retiring from the chairmanship of his Department he was called to California to work on the collections of Bryozoa made by the Hancock Foundation expeditions in Pacific waters from Alaska to Peru. As a result he prepared three noteworthy volumes on his studies. For the excellence of one of these he was awarded the Daniel Giraud Elliot 1950 gold Medal by the National Academy of Science. In 1949 The Ohio State University awarded him the honorary degree of Doctor of Science.

Dr. Osburn lived and practiced a philosophy of life that is nowhere better shown than in the volume of 165 letters sent to him at his retirement containing such expressions as:

". . . your kind and sympathetic attitude has constantly been an inspiration to me."

". . . The kindness of the man himself, his comprehensive soul, his deep, unerring instinct as to each person's goal."

"Sir, I salute you, great teacher, perfect gentleman, naturalist of the old school, and, above all, a true friend."

He lived as he expressed it in a few lines which he himself wrote as he contemplated his retirement:

"What pleases me most, looking back o'er the years,
As the time for retirement now rapidly nears,
Is the progress of students I've worked with, and on,
As out to the world and its work they have gone.
And I like to think, maybe, I've had a small part
In giving a fine bunch of men a good start.
For a teacher there can be no greater reward,
Though his days may be long and his labors be hard,
Than noting the heights which his students attain,
And feeling his efforts have not been in vain."

Dr. Osburn died August 6, and was buried August 9, near his birthplace at Newark, Ohio.

Department of Zoology and Entomology.
The Ohio State University.

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